INTRAPERITONEAL LYSIS OF TUBERCLE BACILLI.*

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Koch[1] observed, in 1891, that a tuberculous guinea pig is more resistant than a normal guinea pig toward inoculation with tubercle bacilli. This observation was not confirmed by Koch's contemporary workers.[2] It was not until seventeen years later, after investigators had learned to avoid conflicting results from anaphylactic phenomena, and mainly as a result of the careful quantitative work of Römer[3] and Hamburger[4] that the observation was finally established. A similar heightened resistance toward reinoculation was demonstrated by Arloing,[5] von Behring,[6] Calmette and Guerin,[7] and others for tuberculous cattle; by Kraus, Gross, and Volk[8] for tuberculous monkeys; and by Römer and Joseph[9] for tuberculous sheep.

Numerous attempts have been made to determine the mechanism of this heightened tubercular resistance. Thus, Römer and Joseph[10] studied the fate of tubercle bacilli injected subcutaneously in tuberculous guinea pigs and found that the bacilli were not destroyed at the site of the reinoculation, but were held in the tissues in a living

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1 Koch, R., *Deutsch. med. Wchnschr.*, 1891, xvii, 101.

2 For a summary of the negative work, see Hamburger, F., *Beitr. z. Klin. d. Tuberk.*, 1909, xii, 261.

3 Römer, P. H., *Ztschr. f. Infektionskrankh. d. Haustiere*, 1909, vi, 393.

4 Hamburger, F., *loc. cit.*

5 Arloing, S., *Compt. rend. Acad. d. sc.*, 1909, cxlix, 962.

6 von Behring, E., *Therap. d. Gegenw.*, 1907, xlviii, 145.

7 Calmette, A., and Guerin, C., *Compt. rend. Acad. d. sc.*, 1910, cli, 32.

8 Kraus, R., and Gross, S., *Centralbl. f. Bakteriol., Orig.*, 1908, xlvii, 298. Kraus, R., and Volk, R., *Wien. klin. Wchnschr.*, 1910, xxiii, 699.

9 Römer, P. H., and Joseph, K., *Beitr. z. Klin. d. Tuberk.*, 1910, xvii, 287.

10 Römer, P. H., and Joseph, K., *ibid.*, 365.

and virulent state, without the production of a tuberculous lesion. They even thought that an actual multiplication of the bacilli took place. The heightened resistance apparently depended upon a power of the tissues to inhibit the pathogenic properties of the bacilli rather than upon a power of destroying them. A rapid lytic process, such as is observed in the immunity of certain other infectious processes, was not demonstrated.

Contrary to this observation Deycke and Much,[11] Much and Leschke,[12] and Kraus and Hofer[13] have recently obtained evidence of lysis by injecting tubercle bacilli into the peritoneal cavities of tuberculous and artificially immunized guinea pigs. They noted a rapid disappearance of the bacilli from the peritoneal fluids, and the appearance in the fluids of atypical and non-staining forms and of granules resembling bacterial fragments.

A rapid decrease in the number of tubercle bacilli in the peritoneal fluids, however, is not necessarily evidence of a destruction of the bacilli, since the decrease might conceivably be brought about by such factors as absorption by the peritoneal lymphatics, or adhesion to the peritoneal surfaces. Non-staining bacilli and granules resembling bacterial fragments are also not necessarily evidence of lysis, since Markl[14] has shown that non-staining forms are often produced in the peritoneal cavities of normal guinea pigs, and Van Giesen[15] has pointed out that granules resembling bacterial fragments can result from tissue disintegration. The evidence cited in support of the belief that lysis takes place is, therefore, not adequate to establish the conclusion beyond doubt. Hence we have been led to review somewhat carefully the experimental evidence in support of this view.

INTRODUCTORY EXPERIMENTS.

In the preliminary experiments eight strains of tubercle bacilli were tested on normal and tuberculous animals. Most of the

[11] Deycke, G., and Much, H., *Beitr. z. Klin. d. Tuberk.*, 1910, xv, 277.

[12] Much, H., and Leschke, E., *Beitr. z. Klin. d. Tuberk.*, 1911, xx, 405.

[13] Kraus, R., and Hofer, G., *Deutsch. med. Wchnschr.*, 1912, xxxviii, 1227; *Wien. klin. Wchnschr.*, 1912, xxv, 1112.

[14] Markl, G., *Centralbl. f. Bakteriol., Orig.*, 1905, xxxviii, 69.

[15] Van Giesen, I., *Med. Rec.*, 1910, lxxviii, 52.

strains were found to undergo marked degeneration when injected into the peritoneal cavities of normal guinea pigs. The bovine strains gave especially rapid involution forms, as early as the end of the first hour after the injection a large number of faintly staining and non-staining bacilli being seen, as well as club-shaped and spindle-shaped organisms and bacilli containing one or more blue staining granules. We finally selected two cultures that gave comparatively few degenerations in normal guinea pigs. These were:

H_{24}, a virulent strain of the human type, originally obtained from Dr. Theobald Smith, and

H_{39}, a slightly more virulent human strain, originally obtained from Dr. E. L. Trudeau.

They were used in the inoculations and tests reported in this paper.

Guinea Pigs.—Half grown guinea pigs were inoculated subcutaneously. By making the inoculations in the axilla, it was found possible to render the guinea pigs tuberculous, without producing tubercular changes in the peritoneal surfaces. A few metastatic nodules usually formed in the lungs and occasionally in the spleen. The liver was usually not involved and the omentum and other peritoneal surfaces usually remained normal. The immunity test was made five or six weeks later by intraperitoneal reinoculation.

In making the intraperitoneal test four cubic centimeters of a carefully filtered or sedimented suspension, containing approximately 80,000,000 tubercle bacilli per cubic centimeter, were injected and a sample of the peritoneal fluid was withdrawn at intervals by means of capillary pipettes, and stained by the Ziehl-Neelsen and Much methods.

The changes observed in the morphology and staining reaction of the withdrawn bacilli were less striking in our hands than those described by Kraus and Hofer. There was usually a more rapid appearance of beaded and club-shaped forms in the tuberculous animal than in the control, as well as bacilli nearly or completely devoid of acid-fast properties and bacilli containing one or more large, blue staining granules. Free granules ("Kügelchen") staining like the intrabacillary granules, on which Kraus and Hofer laid greatest stress, were, however, rarely seen.

The bacilli taken up by the leucocytes were less altered than the extracellular bacilli. Apparently the process of phagocytosis, instead of accelerating the degenerative changes, actually protected the bacilli. In this we incline to agree with Deycke and Much,[16] who look upon phagocytosis as shielding the tubercle bacilli from the curative processes of the body.

We observed with Much and Leschke the most striking difference between the tuberculous and normal guinea pig to be the rapid decrease in the number of bacilli in the peritoneal fluids of the tuberculous animal. In the normal guinea pig the number of bacilli remains large for several days. In the tuberculous guinea pig more than nine tenths of the bacilli disappear within an hour, and all but an occasional bacillus within three hours.

Differences were also noted between the resistance of the normal and tuberculous guinea pigs toward the intraperitoneal reinoculation. The normal guinea pigs all died in from three to four weeks from a fulminating type of visceral tuberculosis, while most of the tuberculous guinea pigs lived from six to twelve weeks. A few of the latter, however, showed immediate toxic symptoms, from which some died within from twenty-four to forty-eight hours in a manner suggestive of the anaphylactic condition described by Römer[17] and Hamburger.[18]

White Rats.—Subcutaneous inoculation of white rats with virulent tubercle bacilli usually produces a slight local induration lasting but a few days. There is generally no subsequent enlargement of the neighboring lymph glands and no formation of secondary lesions in the internal organs.

Tubercle bacilli injected into the peritoneal cavities of normal rats undergo rapid degeneration. Granular, faintly staining and non-staining forms soon appear, both in the extracellular fluids and in the phagocytes. The number of bacilli rapidly decreases, though numerous acid-fast organisms can be found several days after the injections.

In rats previously inoculated the same changes take place, only they are more rapid and more pronounced. Within three hours

[16] Deycke, G., and Much, H., *München. med. Wchnschr.*, 1909, lvi, 1985.
[17] Römer, P. H., *loc. cit.*
[18] Hamburger, F., *loc. cit.*

after the injection the number of bacilli is distinctly less than in the controls, and the bacilli usually completely disappear by the end of twenty-four hours. Inoculated rats, therefore, show the same general phenomenon as tuberculous guinea pigs, though the contrast between the normal and inoculated rats is less striking than that between normal and tuberculous guinea pigs.

Rabbits.—Subcutaneous inoculation of rabbits with virulent human strains produces lesions that are generally strictly local. With the bovine strains the neighboring lymph glands are usually involved and a few secondary tubercles usually form in the lungs.

The reaction of normal rabbits toward intraperitoneal inoculation resembles that of white rats. Phagocytosis is slow, and there is the rapid appearance of degeneration forms. Numerous acid-fast bacilli, however, can be demonstrated several days after the injection.

Tuberculous rabbits vary greatly in their reactions. Phagocytosis is usually slower than in normal rabbits, though in one series it was apparently more rapid. The bacilli slowly decrease in number, and usually disappear within twenty-four hours after the injection. In one of the animals the decrease was exceptionally rapid, a noticeable diminution being observed by the end of one hour. Tuberculous rabbits, therefore, exhibit the same general phenomenon as tuberculous guinea pigs, though here also the contrast between the normal and tuberculous animals is less striking.

Dogs.—Subcutaneous inoculation of dogs with human strains of tubercle bacilli usually produces a slight local induration, disappearing within a few days and giving rise to no glandular or visceral involvement. Bovine strains usually produce a permanent local induration occasionally leading to ulceration. The neighboring lymph glands are usually slightly enlarged, but no visceral lesions result within the first few weeks.

Normal dogs injected intraperitoneally with large doses of the bacilli show a rapid phagocytosis, but almost no immediate change in the morphology and staining reaction of the injected organisms. By the end of four hours phagocytosis is complete, the intracellular bacilli being acid-fast. This condition persists for three or four days, after which the number of leucocytes and bacteria gradually

decreases. Both leucocytes and bacteria usually disappear from the peritoneal fluid by the end of the seventh day.

In the peritoneal cavities of tuberculous dogs phagocytosis is less rapid. A rapid decrease in the number of extracellular bacilli, however, takes place. Within six hours a marked diminution in the number is noticed, and by the end of twenty-four hours the extracellular forms have completely disappeared. By the end of forty-eight hours, the intracellular forms also have disappeared, only an occasional leucocyte being seen containing blue staining granules.

The contrast between the normal and tuberculous dogs is, therefore, similar to that between normal and tuberculous rabbits, being less marked than in guinea pigs.

Monkeys.—In one tuberculous monkey tested by the intraperitoneal method a distinct decrease in the number of tubercle bacilli occurred in the sample withdrawn at the end of one hour and a complete disappearance of the bacilli in the sample withdrawn at the end of two hours. The control monkey showed numerous acid-fast bacilli for several days after the injection. If this experiment is typical, the contrast between normal and tuberculous monkeys is similar to that between normal and tuberculous guinea pigs.

PHENOMENON ANALYZED.

The above experiments show that the phenomenon hitherto interpreted as intraperitoneal lysis can be reproduced in all laboratory animals thus far tried. The phenomenon, however, is more sharply defined in guinea pigs than in other laboratory animals, with the possible exception of the monkey. Guinea pigs were therefore selected for the analysis of the reaction.

The number of bacteria present in an aspirated sample of peritoneal fluid depends upon several factors, among which are the total volume of the peritoneal fluid, the amount of bacterial absorption by the peritoneal lymphatics, and the amount of bacterial adhesion to the peritoneal surfaces.

Volume.—When suspensions of tubercle bacilli are injected into the peritoneal cavity of normal guinea pigs, a rapid absorption of the injected fluid takes place (AN, figure 1). One half of the fluid

disappears by the end of three hours, and the peritoneal cavity becomes practically dry by the end of ten hours.

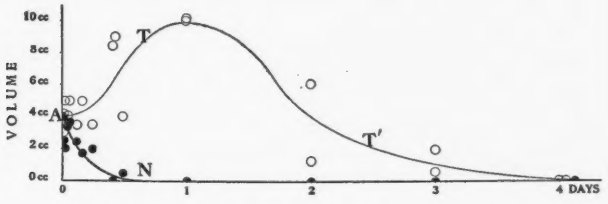

FIG. 1. VOLUME OF PERITONEAL FLUID. Uniform amounts (4 c.c.) of a suspension of tubercle bacilli were injected into the peritoneal cavities of a series of guinea pigs. The volume of the peritoneal fluid was afterwards determined at autopsy. AN = approximate changes in the volume of the peritoneal fluid in normal guinea pigs; ATT′ = approximate changes in tuberculous guinea pigs.

In tuberculous guinea pigs, in contrast, there occurs at first an increase in the volume of the peritoneal fluid (AT). The volume may be doubled during the first ten hours, and the increase usually continues during the first twenty-four hours. Later the volume gradually decreases (TT′), and the peritoneum usually becomes dry by the end of the fourth day.

A rapid decrease in the number of tubercle bacilli would therefore be expected in samples of peritoneal fluid withdrawn from tuberculous guinea pigs, due solely to this initial increase in the volume of the peritoneal fluid, while an increase in the number of bacilli would be expected in normal guinea pigs due to the volumetric decrease.

Leucocytes.—If four cubic centimeters of Ringer solution are injected into the peritoneal cavity of a guinea pig and a sample of the peritoneal fluid is immediately withdrawn, this sample will usually be found to contain approximately 4,500 leucocytes per cubic millimeter. Or, calculating the total number of leucocytes free in the peritoneal cavity, allowing one cubic centimeter as the original volume of the peritoneal fluid before the introduction of the Ringer solution, we obtain about 22,000,000 as the total number of leucocytes present. Differential counts show approximately 10 per cent. polymorphonuclear leucocytes, 50 per cent. large mononuclear cells, and 40 per cent. lymphocytes.

Immediately after the introduction of the suspension of tubercle bacilli there usually takes place a rapid decrease in the number of

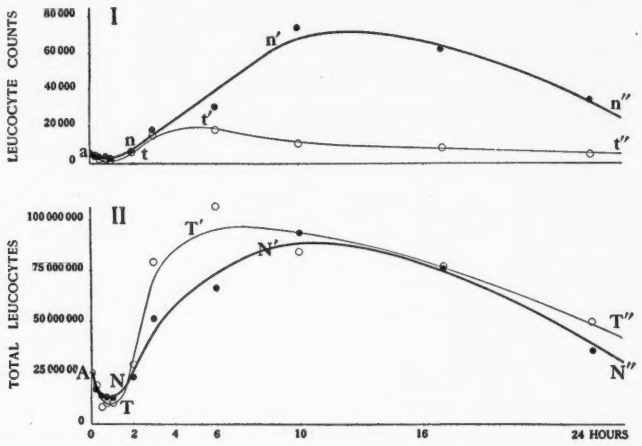

FIG. 2. PERITONEAL LEUCOCYTES. I = changes in the leucocytic count per cubic millimeter of peritoneal fluid, following the introduction of tubercle bacilli. ann' n'' = normal guinea pig; att' t'' = tuberculous guinea pig.

II = calculated changes in the total number of leucocytes free in the peritoneal fluid of the above animals. ANN' N'' = normal guinea pig; ATT' T'' = tuberculous guinea pig.

these leucocytes (I, figure 2). In normal guinea pigs (an, figure 2) the average leucocyte count falls within fifteen minutes to about 2,500 per cubic millimeter, and reaches 2,000 per cubic millimeter by the end of one hour. In tuberculous guinea pigs an even more rapid fall takes place (at, figure 2), the average count being reduced to 1,500 per cubic millimeter within thirty minutes. In particular instances counts as low as 150 per cubic millimeter have been observed.

This initial fall is due to an agglutination of the leucocytes and an adhesion of the resulting leucocytic masses to the omentum and adjacent gastrohepatic membranes. Agglutinated masses are occasionally seen in samples of the peritoneal fluid withdrawn at the end of fifteen minutes. At autopsy the omentum is found studded with these masses, many of which are sufficiently large to be visible

to the naked eye. The leucocytes thus mechanically removed from the peritoneal fluids are mainly the large mononuclear and polymorphonuclear cells (figure 3).

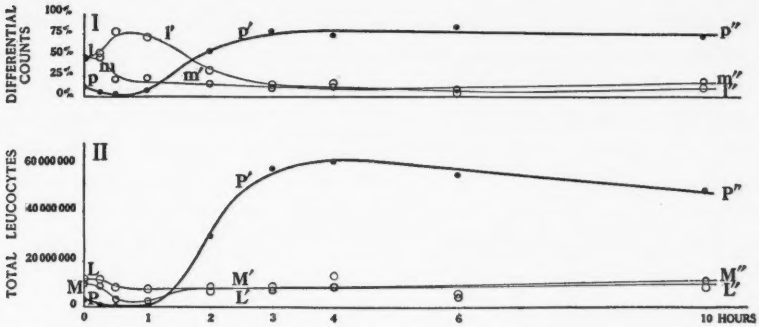

FIG. 3. TYPE OF LEUCOCYTES. I = changes in the differential leucocytic counts; composite picture obtained by taking the average count from four normal and four tuberculous guinea pigs. pp′ p″ = polymorphonuclear leucocytes; mm′ m″ = large mononuclear cells; ll′ l″ = lymphocytes.

II = calculated changes in the total number of free peritoneal leucocytes of each type. PP′ P″ = polymorphonuclear leucocytes; MM′ M″ = large mononuclear cells; LL′ L″ = lymphocytes.

Following this initial drop, there is a secondary rise in the leucocytic count (I, figure 2), the original number as a rule being restored within two hours, and the number increasing to three or four times the original number by the end of the third hour. In tuberculous guinea pigs this increase tends to reach its maximum by the end of the sixth hour (tt′, figure 2). In normal guinea pigs the increase continues for at least ten hours (nn′, figure 2), when the count may be from ten to fifteen times the original number.

This secondary increase is not due to a breaking up of the leucocytic masses adherent to the omentum. In normal guinea pigs a part of the increase is due to a diminution in the volume of the peritoneal fluid; but that an actual increase in the leucocytes occurs in both normal and tuberculous animals is brought out by calculating the changes in the total number of leucocytes free in the peritoneal fluids (II, figure 2). The leucocytes thus added to the perito-

neal fluid are for the most part polymorphonuclear cells (figure 3).

Tubercle Bacilli.—To determine the changes in the number of tubercle bacilli, smears were stained by the Ziehl-Neelsen and Much methods, and the number of bacteria per hundred leucocytes were counted. From this number the number of bacteria per cubic millimeter, and the total number free in the peritoneal cavity, were calculated. The changes in the total bacterial content, in a typical case, are shown in figure 4.

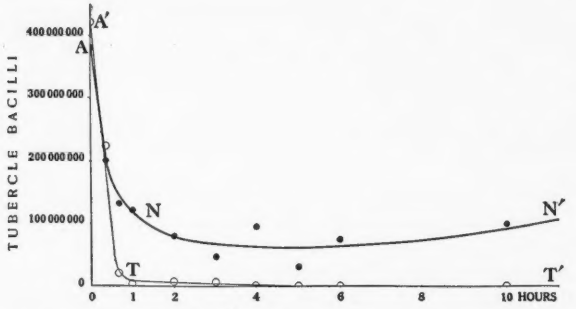

FIG. 4. CHANGES IN THE TUBERCLE BACILLI. Changes in the total number of tubercle bacilli free in the peritoneal fluid. ANN′ = normal guinea pig; A′ TT′ = tuberculous guinea pig.

In this experiment approximately 400,000,000 tubercle bacilli were injected into the peritoneal cavity of each animal. In the normal guinea pig over 50 per cent. of the bacteria disappeared from the peritoneal fluid within twenty minutes, and 75 per cent. within the first hour. The number remained approximately 100,000,000 in this animal from that time till the end of the experiment.

In the tuberculous guinea pig the same initial decrease took place, the number falling 50 per cent. during the first twenty minutes. Here, however, the rapid decrease continued, the number being reduced to about 1 per cent. by the end of the first hour, and bacilli completely disappearing by the end of the fourth hour. In most of the experiments, however, the contrast between the normal and tuberculous guinea pigs was not as great as this. Numerous bacilli were occasionally demonstrable in the peritoneal fluids of the tuber-

culous animals, five or six hours after the injection, particularly in cases in which the abdomen had been thoroughly massaged immediately before withdrawing the peritoneal sample.

Distribution.—An examination of the abdominal cavity of a normal guinea pig, made two hours after the injection, shows numerous tubercle bacilli in the scrapings from all the peritoneal surfaces. The peritoneal surfaces of a tuberculous guinea pig, in contrast, at this time usually show few tubercle bacilli except upon the omentum. On the omentum numerous bacilli are found, adherent for the most part to the leucocytic masses previously described.

Later examinations, as, for example, at the end of twenty-four hours (table I), give even more marked contrasts. In the normal guinea pig numerous bacilli are demonstrable on all the peritoneal surfaces, both by the Ziehl-Neelsen and the Much methods. In the tuberculous guinea pig the peritoneal surfaces, with the exception of the diaphragm, the omentum, and the gastrohepatic membranes, are commonly free from tubercle bacilli, as determined by the Ziehl-Neelsen method. Numerous granules, however, can be demonstrated on the various surfaces by the Much method. After forty-eight hours, the contrast is still more striking, since by this time most of the Much granules have disappeared.

TABLE I.

Peritoneal Distribution.

Smears made from the peritoneal surfaces of normal and tuberculous guinea pigs, twenty-four and forty-eight hours after the injection of tubercle bacilli. + = bacilli, . = granules, — = no examination.

Surface.	24 hours.				48 hours.			
	Tuberculous.		Normal.		Tuberculous.		Normal.	
	Ziehl.	Much.	Ziehl.	Much.	Ziehl.	Much.	Ziehl.	Much.
Peritoneal fluid.....	o	.	+	+.	o	o	++	+++
Parietal peritoneum	o	....	+++	++..	o	o	++++	++++
Intestine...	o	o	+++	++..	o	o	++	+++
Mesentery..	o	—	++	++..	o	o	++	+++
Stomach ...	o	—	+	−	o	o	++	+++
Liver.....	o	—	++++	+++...	o	o	++	+++
Diaphragm.	++	++..	+++	++..	+	+.	++	+++
Omentum ..	++	+++..	+++	+++..	++	++	++++	++++

Autopsies made at the end of four or five weeks usually confirm these findings. While all peritoneal surfaces of the normal guinea pig are usually closely studded with miliary tubercles, most of the surfaces of the tuberculous animal are usually free from macroscopic lesions, at most half a dozen isolated tubercles being visible. The omentum, however, is always severely affected.

Agglutination.—One of the factors that assists in confining the tuberculous process to the omentum is an adhesion of the injected bacilli to the leucocytes. The agglutinated leucocytes seen in the early samples of peritoneal fluid usually carry with them from ten to a hundred tubercle bacilli adherent to each leucocyte.

Absorption.—A second mechanical factor that conceivably assists in freeing the peritoneal cavity of tubercle bacilli is absorption by the peritoneal lymphatics. An attempt was made to estimate the importance of this factor.

Through the kindness of Dr. Dochez a strain of pneumococcus was secured, which, injected into the peritoneal cavity of a normal guinea pig, undergoes comparatively little change during the first three hours. When a mixture of tubercle bacilli and this pneumococcus was injected into the peritoneal cavity of a tuberculous guinea pig, the tubercle bacilli usually disappeared from the fluid within thirty minutes, while the number of pneumococci was not appreciably reduced. This effect cannot be due to a mechanical absorption of the peritoneal fluid, since, if due to such a cause, the reduction in the number of tubercle bacilli and pneumococci would be proportional. Peritoneal absorption, therefore, plays a small part in the immediate decrease in the number of tubercle bacilli.

Granules.—The existence of mechanical factors tending to remove the bacilli from the peritoneal fluid and to cause their accumulation on the omentum, renders the proof or disproof of lysis difficult. The only direct evidence of lysis we have thus far obtained was from smears made from tuberculous guinea pigs, about thirty minutes after the introduction of the bacilli. In one case numerous acid-fast granules were seen, unmistakable fragments of tubercle bacilli. Non-acid-fast granules (Much granules) are usually seen in both normal and tuberculous animals, and cannot be taken as evidence of specific lysis.

Quantitative Analyses.—A quantitative determination of the residual bacteria in the peritoneal cavity was attempted. To make this determination, the peritoneal surfaces were repeatedly scrubbed with citrate solution and afterwards with 5 per cent. antiformin. The washings were united and allowed to stand over night in 10 per cent. antiformin. In the morning the undissolved tubercle bacilli were thrown down by centrifugation, an equal volume of alcohol being added to favor sedimentation. The alcohol forms a fine albuminous precipitate, which tends to hold the sedimented bacteria together. The sediment was washed with 0.2 per cent. hydrochloric acid and thoroughly emulsified in a known volume of serum, the serum being added to cause an adhesion of the bacteria to the microscopic slide. In estimating the number of the bacteria, homologous leucocytes were added to the suspension and the number of these leucocytes per cubic millimeter was determined, together with the average number of tubercle bacilli per 100 leucocytes.

By this method it was found that the bacteria could be recovered practically quantitatively from the peritoneal cavities of normal guinea pigs from one and one half to two hours after the injection. In a few determinations an apparent increase in the number of bacilli was noted, the average recovery being 106 per cent. of the original number injected. In tuberculous guinea pigs, in contrast, the analysis uniformly showed a considerable decrease in the number of the bacilli, the average recovery being but 65 per cent. of the original number injected. A definite decrease in the number of tubercle bacilli, therefore, does take place in the peritoneal cavity of tuberculous guinea pigs, independent of the apparent decrease due to changes in peritoneal distribution.

Lysis in Vitro.—That this decrease is due to an actual lysis was shown by experiments with isolated peritoneal tissues. If the small intestine of a fasting normal guinea pig is tied off, transferred to a warmed test-tube containing a known number of tubercle bacilli, and the preparation incubated from three to ten hours, no diminution in the number of tubercle bacilli can be demonstrated by the antiformin method (table II). There is often even a slight apparent increase in the number of bacilli thus recovered. With the intestine of a tuberculous guinea pig, however, there is uniformly

obtained a considerable decrease in the number of the bacilli, the average recovery being only 55 per cent. of the original number placed in the test-tube. In one experiment only 14 per cent. of the original number was thus recovered.

TABLE II.

Lysis of Tubercle Bacilli in Vitro.

A uniform number of tubercle bacilli were exposed at 37° C. to the action of peritoneal tissues *in vitro* for the lengths of time specified. The residual bacteria were determined by the antiformin method (Ziehl-Neelsen stain). The culture used in the tests was a six weeks' glycerin agar growth of H_{24}.

Time.	Bacilli recovered.			
	Normal.		Tuberculous.	
3½ hrs.	122%		62%	
4 hrs.	97%	Average	98%	Average
4½ hrs.	91%	109%	30%	73%
5 hrs.	127%		93%	
9 hrs.	100%		44%	
10 hrs.	107%	100%	60%	38%
10 hrs.	97%		33%	
11 hrs.	97%		14%	
Average		105%		55%

The number of tubercle bacilli destroyed by these tissues *in vitro* apparently depends upon the age and virulence of the culture tested. With a fresh culture of virulent organisms fewer bacilli are destroyed than with an older culture of a less virulent strain.

MECHANISM OF THE LYSIS.

The above studies clearly indicate that an actual lysis of tubercle bacilli is possible in tuberculous animals. The question now arises as to the mechanism of this tuberculolysis.

Circulating Antibodies.—The first factor examined was the possibility that lysis was due to substances present in the circulating blood. Tests were therefore made with sera, peritoneal fluids, and leucocytes of tuberculous and normal guinea pigs. No distinct lytic effects could be produced *in vitro* with any of these substances, either when tested alone, or in combination. Involution forms of the tubercle bacillus were often seen after exposure to the fluids

and cells of tuberculous animals; but similar involutions were produced by the products of normal guinea pigs, and often appeared in control suspensions in Ringer solution.

Aside from a slight agglutinating action occasionally seen with the sera of tuberculous guinea pigs, the only evidence of circulating antibodies thus far obtained was in certain quantitative determinations with large amounts of freshly drawn defibrinated blood. If a large amount of normal blood is mixed with a small number of tubercle bacilli, and the mixture incubated for twelve hours, a slight apparent increase (10 to 20 per cent.) in the number of tubercle bacilli is observed by the antiformin method. When the blood of tuberculous guinea pigs is used, this increase is not noted, suggesting a possible inhibition of multiplication by the tuberculous blood.

Passive Immunization.—The second factor examined was the possibility that the body fluids contain tuberculolytic substances which require for their action the coöperation of the fixed cells of the body. To test this possibility sera and peritoneal fluids of tuberculous guinea pigs were introduced into the peritoneal cavities and circulatory system of normal guinea pigs, and the guinea pigs subsequently tested with intraperitoneal injections of tubercle bacilli. No lytic phenomena, however, were detected, though in one experiment a slight intraperitoneal agglutination was observed.

Transfusions.—On account of this negative result, the effect of transfusing large quantities of unaltered blood was tested. The initial transfusions were made with dogs and rabbits. In these animals it is easy to pass large quantities of blood from one animal to another, by using ordinary paraffined cannulæ and rubber tubing. By selecting animals of different sizes, or by using two or more tuberculous animals as the source of the blood, one can readily replace practically the entire volume of blood in the normal animal with that of tuberculous animals. The transfused animals were subsequently tested by intraperitoneal injections of tubercle bacilli, but in no case was lysis detected.

Transfusions were then made with guinea pigs, the guinea pigs being tested from twenty hours to three days after the operation. Here also in no instance was lysis observed. The subsequent histories of the transfused guinea pigs confirm this finding, the trans-

fused animals and the normal controls all dying, about four weeks later, from a fulminating type of visceral tuberculosis. The autopsies showed identical lesions in the two groups.

Fixed Cells.—It would appear from these experiments that the power of intraperitoneal lysis of tubercle bacilli does not depend primarily upon substances present in the circulating blood. Hence it would seem to follow that it depends largely or wholly upon certain properties of the local fixed peritoneal cells.

This conclusion agrees with the elaborate theory of partial antibodies recently put forth by Deycke and Much,[19] who state that they have obtained evidence, from complement deviation and anaphylactic studies, that tuberculous animals possess four distinct groups of partial antibodies, directed respectively against the neutral fats, the lipoids, the proteids, and the specific toxins of the tubercle bacillus. They also believe that some of these antibodies exist as circulating antibodies, while others are held in the fixed cells. Our experiments do not exclude the possibility that the fundamental lytic power of the fixed peritoneal cells is assisted or supplemented by circulating antibodies.

TUBERCULAR IMMUNITY.

The phenomenon of intraperitoneal lysis of tubercle bacilli is of importance because of the light it may eventually throw upon the nature of tubercular immunity. It would seem probable that the lytic property is not confined to the peritoneal cells alone and that it may also be acquired by other fixed cells.

Just how widely distributed throughout the body the cells are that acquire this power is a problem on which we are at present engaged. If the cells are found to be widely distributed it would suggest that tubercular immunity is essentially a specific adaptation of fixed local cells to destroy tubercle bacilli brought into immediate contact with them.

SUMMARY.

1. Tubercle bacilli injected into the peritoneal cavities of tuberculous guinea pigs, rats, rabbits, dogs, and monkeys, rapidly disappear from the peritoneal fluids, while persisting in the peritoneal fluids of normal control animals.

[19] Deycke, G., and Much, H., *München. med. Wchnschr.*, 1913, lx, 119, 190.

2. This disappearance is in part due to an adhesion of the injected bacilli to the peritoneal leucocytes and a fixation of the leucocytes on the omentum.

3. The injected tubercle bacilli can be recovered quantitatively from the peritoneal cavities of normal guinea pigs from one and one half to two hours after the injection, while from tuberculous guinea pigs only 65 per cent. of the bacilli can be recovered at this time.

4. Isolated peritoneal tissues from tuberculous guinea pigs have the power of destroying tubercle bacilli *in vitro*.

5. A second factor reducing the number of tubercle bacilli free in the peritoneal fluid is therefore an actual lysis of the bacilli.

6. The intraperitoneal lysis is not due solely to substances present in the circulating fluids, since the phenomenon cannot be produced by these fluids *in vitro*, and since a lytic power cannot be passively conferred even by a direct transfusion of blood from tuberculous to normal animals.

7. The intraperitoneal lysis is apparently due to specific changes in the fixed peritoneal cells of the tuberculous animals.

FURTHER EXPERIMENTAL STUDIES IN TETANY.*

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In a paper read before the German Pathological Association at the meeting in Strassburg in 1912, I showed that the galvanic hyperexcitability of the nerves, which is a characteristic feature of tetany, is due to a change in the blood following the destruction of the parathyroid glands. It was demonstrated that after section of a nerve during tetany the isolated extremity remained hyperexcitable, and that such an isolated extremity became hyperexcitable if extirpation of the parathyroids was carried out after the cutting of the nerve. Then by connecting the blood vessels of an animal in the height of tetany with those of the leg of a normal animal so that the peripheral portion of the nerves of the limb of the normal animal were bathed in tetany blood, it was demonstrated that an excitability identical with that found during tetany appears very quickly in those nerves, and as quickly gives place to the normal conditions, when the femoral vessels are reunited with their stumps so that the nerves are again supplied with normal blood. In the same way the flooding of one leg of an animal in tetany with normal blood reduces the excitability of the nerves of that leg to the normal while the rest of the animal is in tonic and clonic convulsions.

The restoration of the femoral vessels to their original connections, so that the animal's own blood again flows through the leg, brings that extremity back to the condition of the others, and hyperexcitability of the nerves and twitching are again seen there. Similarly the blood removed from an animal during the convulsions of tetany and defibrinated may be perfused through the vessels of the amputated leg of a normal animal, whereupon its nerves become

* Received for publication, July 2, 1913.

hyperexcitable. If normal blood and tetany blood be used alternately in this perfusion the nerves will become correspondingly normally excitable or hyperexcitable. That this is not due to any mechanical disturbance of the circulation was shown by numerous experiments.

It is evident, therefore, that after the extirpation of the parathyroid glands there appears gradually a profound change in the blood which makes it capable of acting upon the nerves or their terminations in such a way as to make them respond to electrical stimuli far more rapidly than is normal. Doubtless this statement may be applied to the nerve cells of the spinal cord and brain as well as to those of the sympathetic system, but no ready means of demonstrating this has been devised.

It seemed, therefore, that since one might obtain in a bottle blood capable of producing this characteristic change in the excitability of the nerves, it should be possible to determine precisely the nature of the change and in that way throw light more directly upon the function of the parathyroid gland. For if it is the function of that gland to prevent the appearance of this change in the blood, it should be possible to penetrate further into the activities of this typical organ of internal secretion by discovering what chemical changes have occurred in the blood when the gland is destroyed.

Some years ago upon finding that tetany in animals and man could be interrupted at once by injections of a salt of calcium, Voegtlin and I suggested and upheld the idea that the glands in question might control the metabolism of calcium so that after their loss a serious disturbance in the calcium content of the blood might take place. This view was strengthened by the discovery that the blood of animals during tetany is distinctly poor in calcium and not especially weakened by the fact that other substances such as strontium and magnesium will also cut short the tetany. Undoubtedly when administered in this way the effect of such substances is to decrease the excitability of the nerves, and it is plain, therefore, that the effect would be the same no matter what substance might be responsible through its absence for the hyperexcitability.

The evidence at our command has not been sufficient to enable us to say with certainty what is the nature of the essential change in

the blood in tetany, and this is the problem which I have kept before me in the work of the past winter. It is known that the blood drawn from an animal in tetany differs from normal blood in its ability to cause hyperexcitability of the nerves with which it comes into contact, but it is still a matter of great difficulty to analyze this blood and to determine directly what change in its composition is responsible for its new properties. Any chemical procedure, even one so harmless, to all appearances, as the addition of Ringer's solution, will unfit the blood for perfusion since the nerves quickly die and lose their excitability when bathed with anything but fresh intact blood. Very indirect methods must therefore be used, and as yet it cannot be said that the matter is cleared up.

Three possibilities presented themselves for study, although of course there may be many more.

(1) The blood in tetany may contain a substance somewhat like strychnia in its mode of action, which may affect the nerves directly and increase their excitability.

(2) The blood may lack the normal amount of calcium and in that way cause the nerves to become hyperexcitable.

(3) The poison circulating in the blood may be of the nature of an oxalate and act by precipitating and thus withdrawing calcium from the tissue.

The simplest way in which this group of possibilities might be tested ought to be through the intravenous administration of an extract of the parathyroid gland. If this can counteract the effect of destruction of the parathyroids it must act upon the substances indicated in the first and third of the above possibilities, since the extract of the parathyroid could hardly contain enough calcium to be curative in the case of the second possibility. If then the addition of parathyroid extract to the tetany blood used in perfusion should eliminate its exciting effect, there must be a strychnia-like poison there, or else a substance capable of throwing out of action the calcium normally present. If not it would seem most probable that the blood had been simply deprived of its calcium.

Several experiments of this sort were made, of which the following will be recorded.

Dog 1,250.—(Text-figure 1.) Excitability normal. Perfusion with tetany blood started at 12.50 P. M. Parathyroid extract (cow) added in small amounts (3 to 8 c.c.) at short intervals from 1.10 to 2.55 P. M., in all 40 c.c. to about 1,000 c.c. of blood.

	12.30	1.00	1.10	1.15	1.20	1.25	1.30	1.35	1.45	1.50	2.00	2.15	2.20	2.30	2.40	2.50	3.00	3.10	3.20	3.25	3.30	3.35
KC[1]. . . .	0.5	0.5	0.1	0.05	0.1	0.1	0.05	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.5	0.1	0.1	0.1	0.2	0.2	0.1	0.4
KO. . . .	Negative at 3.0	1.6	0.6	0.4	0.4	0.4	0.6	0.8	1.0	0.8	0.8	1.0	2.2	0.8	1.0	0.8	0.8	0.8	1.0	0.8	1.2	3.0
AC.	0.6	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.2	0.4	0.5	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.8
AO.	2.6	0.8	0.6	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.6	0.8	0.4	0.5	0.3	0.5	0.5	0.5	0.5	0.5	1.4
			ă		ă	ă		ă		ă		Normal blood	ă			ă			Calcium chloride injected			

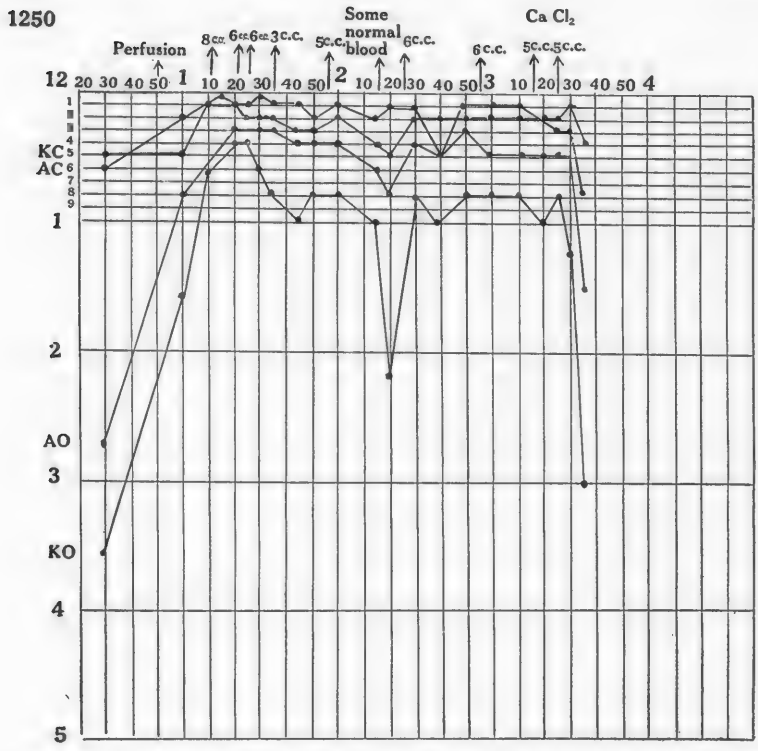
Parathyroid extract injected at *a*.

Dog 1,308.—(Text-figure 2). Normal excitability. Perfusion with normal blood complete at 5.10 P. M. Tetany blood started at 5.30 P. M. 25 c.c. of parathyroid extract injected at intervals in doses of 5 c.c.

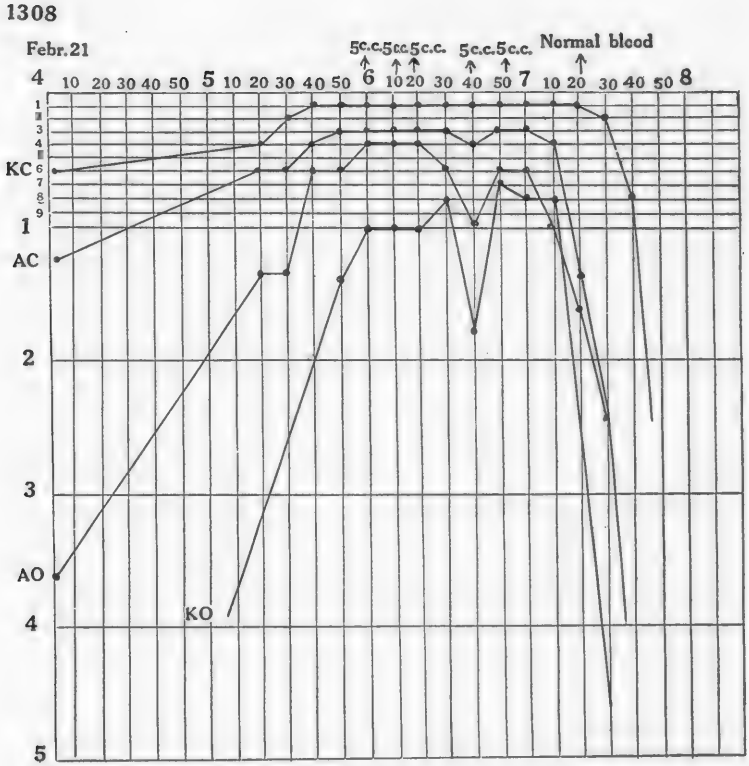
	4.00	5.10	5.20	5.30	5.40	5.50	6.00	6.10	6.20	6.30	6.40	6.50	7.00	7.10	7.20	7.30	7.35	7.45
KC . . .	0.6	Perfusion complete.	0.4	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.8	Negative at 4
KO . . .	Negative at 4.0		Negative at 5.0	Negative at 5.0	Tetanus at 2.0	1.4	1.0	1.0	1.0	0.8	1.8	0.7	0.8	0.8	Negative at 3.0	Negative at 5.0	Negative at 5.0	——
AC . . .	1.2		0.6	0.6	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.4	1.4	2.4	4.0	——
AO. . . .	3.6		1.4	1.4	0.6	0.6	0.4	0.4	0.4	0.6	1.0	0.6	0.6	1.0	1.6	2.4	4.0	5.0
							ă	ă		ă		ă						

Parathyroid extract injected at *a*.

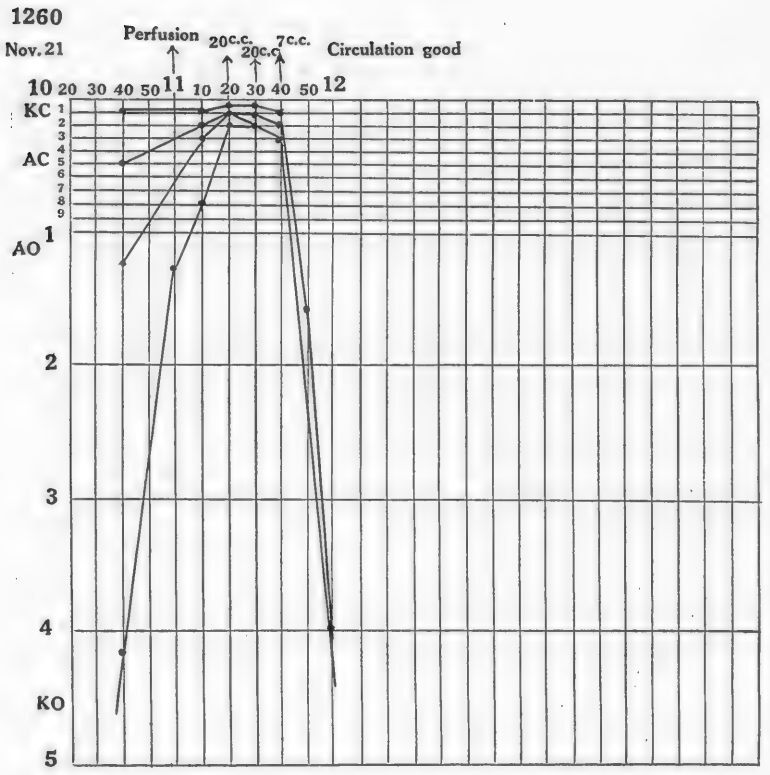
[1] KC = kathode closing; AO = anode opening, etc.



TEXT-FIG. 1. Perfusion of leg with tetany blood. Parathyroid extract added at intervals.



TEXT-FIG. 2. Normal leg perfused with tetany blood plus parathyroid extract, later with normal blood.



TEXT-FIG. 3. Perfusion of leg with tetany blood. Large doses of parathyroid added. Nerves injured.

The parathyroid extract seems to have little effect unless a large dose is given, when it seems capable of killing the nerves, as is shown in dog 1,260. The circulation was perfect throughout.

Dog 1,260.—(Text-figure 3.) Normal excitability. Perfusion at 11 A. M. with tetany blood to which there was added parathyroid extract as follows: 20 c.c. at 11.20 A. M., 20 c.c. at 11.30, and 7 c.c. at 11.40.

	10.00	11.00	11.10	11.20	11.30	11.40	11.50	12.00
KC.....	0.1	Perfusion complete.	0.1	0.05	0.05	0.1	1.6	4.0
KO.....	Negative at 4.0		1.2	0.2	0.2	0.3	1.6	4.0
AC.....	0.5		0.3	0.1	0.1	0.2	1.4	4.0
AO.....	1.2		0.3	0.1	0.2	0.5	1.4	4.0
					a	*a*	*a*	

Parathyroid extract injected at *a*. Circulation good.

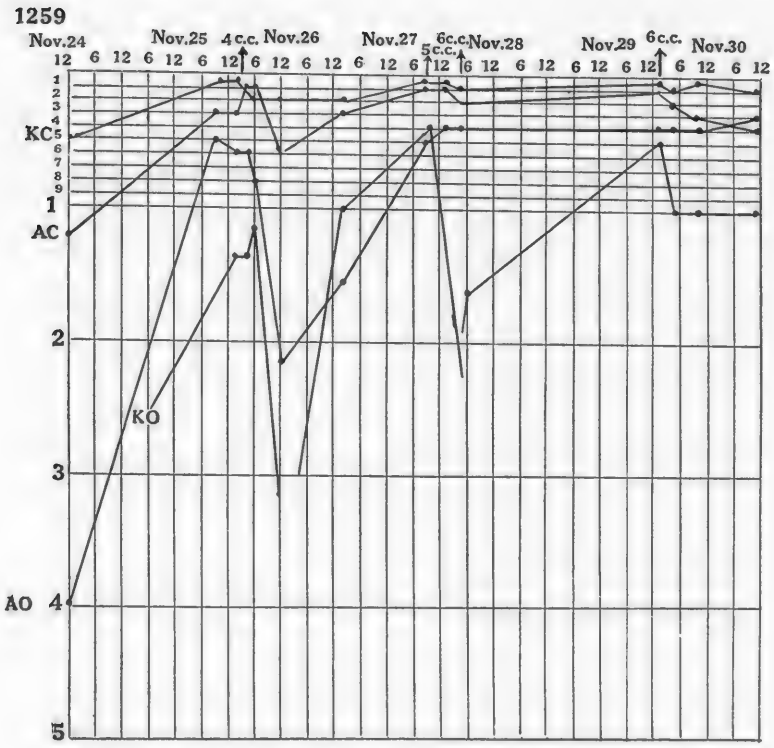
It was thought probable that since such tests last through a relatively short time their results might be untrustworthy, and, further, that in case the action of the parathyroid were directed against an oxalate-like substance there would be very little source for fresh calcium from the small amount of bony and other tissue in one extremity. For these reasons it seemed better to determine the effect of direct injections of parathyroid extract into the jugular vein of an animal in the height of tetany. Ten such experiments were made, all with practically the same result, so that only two or three will be recorded here. The extract of parathyroid glands used was made in some instances from the glands of the ox and extracted with Ringer's solution after being ground up fine with sand. The slightly blood-tinged filtrate from this suspension was used without any further treatment. In some of the experiments glycerin was added to the Ringer's solution which was used in the extraction. In other cases, since it seemed possible that the extract from ox parathyroid might be inefficient in the tetany of the dog, an extract was made from dog's parathyroids which, fortunately, were obtainable in large numbers. The same methods of extraction were used, and in one case 128 glands were extracted in fifty cubic centimeters, in the other seventy glands.

Such extracts, as well as the nucleoproteid precipitated from them by slight acidulation, have been used clinically to alleviate the symptoms of tetany and we have reported the apparent good effect in cases of experimental tetany when administered intravenously or subcutaneously. When they have been given by mouth I have not obtained any special effect, but that is the method employed in treating human beings, and beneficial results have been reported. The course of experimental tetany is notoriously irregular and remissions in its severity are common. Nevertheless, in earlier experiments I have been convinced, as I have in this later series, and as many others have reported, that distinct prolongation of the animal's life could be attained by the intravenous injection and that the twitchings and convulsions could be definitely diminished or even stopped. All these experiments were very rough, however, and no exact measure of the effect could be recorded. Now when we can measure exactly at least one feature of the disturbance, it is surprising to find that even enormous doses of parathyroid extract given intravenously do very little to lower the increased excitability of the nerves. It is true that in some cases there may be a temporary lowering but it is ephemeral, and in most instances all the readings remain up in the tetany level. Yet the animal shows a much decreased tendency to outspoken tetany and lives a good deal longer, so that the idea obtrudes itself that there may be another factor of importance which is different from that which produces the nervous hyperexcitability, or that the ganglion cells are more readily affected by the extract than the nerve terminations. The character of this effect will become clear in the following protocols and curves, but from them, and taking into consideration the enormous doses given directly into the vein, it seems questionable that the mere administration of small doses by mouth should really have the specific effects so often attributed to it.

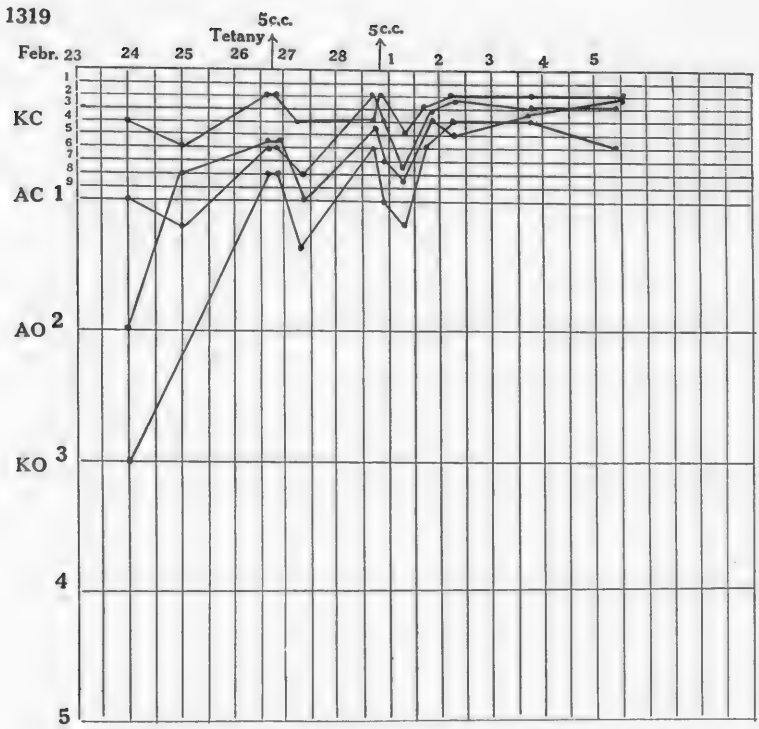
Dog 1,259.—(Text-figure 4.) Parathyroids removed November 24. Tetany not outspoken. Injections of extract of cow parathyroid (fifteen glands to 20 c.c. of Ringer's solution) as shown below.

	Nov. 21.	Nov. 24.	Nov. 25.						Nov. 26.	Nov. 27.				Nov. 29.				Nov. 30.
	12.00	12.00	11.00	2.30	3.20	5.00	5.45	12.00 M.	3.30	10.30	12.45	5.30	5.45	2.45	3 30	4.45	9.00	1.00
KC.....	0.5	Operation.	0.05	0.05	0.05	0.1	0.2	0.2	0.2	0.05	0.05	0.1		0.05		0.1	0.05	0.1
KO.....	Negative at 3.0		Negative at 3.0	1.4	1.4	1.4	1.2	Negative at 3.0	1.0	0.4	Negative at 6.0	1.6		0.5		1.0	1.0	1.0
AC.....	1.2		0.3	0.3	0.1	0.2	0.2	0.6	0.3	0.1	0.1	0.2		0.1		0.2	0.3	0.2
AO.....	4.0		0.5	0.6 ă	0.6	0.6	0.8	2.1	1.2	0.5 ă	0.4	0.4		0.4 ă		0.4 ă	0.4	0.4

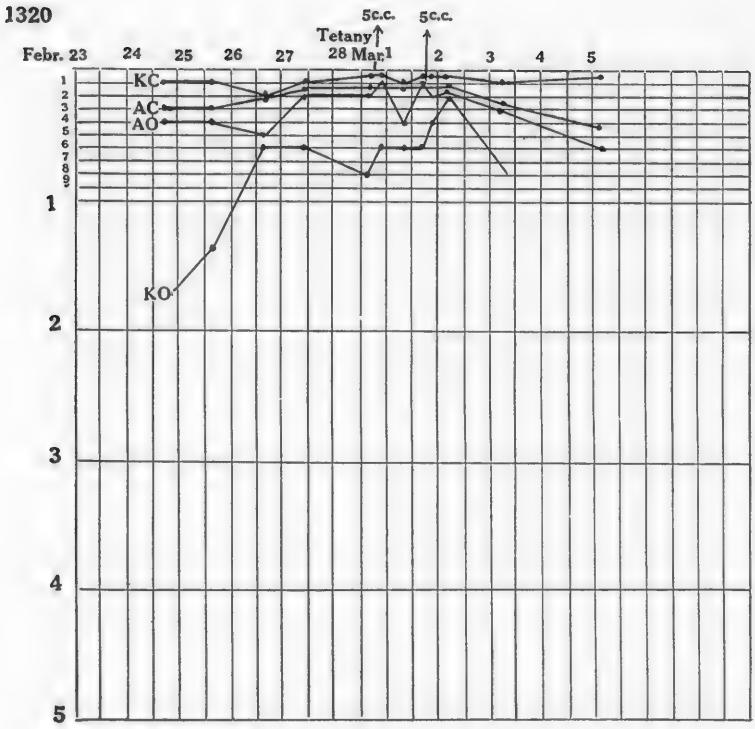
Parathyroid extract injected at *a*. Tetany on November 27 and 29.



TEXT-FIG. 4. Indefinite tetany. Injections of parathyroid extract.



TEXT-FIG. 5. Injections of parathyroid extract.



TEXT-FIG. 6. Tetany. Injections of parathyroid extract.

Dog 1,319.—(Text-figure 5.) Parathyroids removed February 23. Violent tetany February 26. Given two small doses of extract. Tetany disappeared or remained indefinite. Excitability decreased slightly after each injection but remained high.

	Feb. 24.	Feb. 25.	Feb. 26.		Feb. 27.	Feb. 28.		Mar. 1.		Mar. 2.	Mar. 3.	Mar. 4.	Mar. 5.
		2.40	3.45	4.30	11.30	4.00	5.00	10.00	2.30	11.30	5.15		12.20
KC.....	0.4	0.6	0.2	0.2	0.4	0.4	0.2	0.6	0.3	0.2	0.2		0.2
KO.....	3.0	Negative at 4.0	0.8	0.8	1.4	0.6	1.0	1.2	0.6	0.4	0.4		0.6
AC.....	1.0	1.2	0.6	0.6	0.8	0.2	0.4	0.8	0.3	0.2	0.3		0.3
AO.....	2.0	0.8	0.6	0.6	1.0	0.4	0.6	0.8	0.4	0.5	0.4		0.2
					a		*a*						

Parathyroid extract injected at *a*.

Dog 1,320.—(Text-figure 6.) Parathyroids removed February 23. Tetany distinct but not violent on February 28. Rather indefinite throughout the rest of the ten days' course, despite the high excitability. Two small doses of extract given without obvious result.

	Feb. 24.	Feb. 25.	Feb. 26.	Feb. 27.	Feb. 28.			Mar. 1.			Mar. 2.	Mar. 3.	Mar. 4.	Mar. 5.
					3.50	4.15	5.00	10.00	3.00	5.15				
KC...	0.1	0.1	0.2	0.1	0.05		0.05	0.1	0.05	0.05	0.05	0.1		0.05
KO...	—	1.4	0.6	0.6	0.8		0.6	0.6	0.6	0.4	0.2			Indefinite at 3.0
AC...	0.3	0.3	0.2	0.1	0.1		0.1	0.2	0.1	0.1	0.1	0.3		0.4
AO...	0.4	0.4	0.5	0.2	0.2		0.1	0.4	0.1	0.2	0.2	0.3		0.6
						a			*a*					

Parathyroid extract injected at *a*.

Dog 1,326.—Parathyroids removed March 17. Tetany distinct on March 19 and twitchings continued, sometimes amounting to definite tetany. Killed March 24. Parathyroid extract given at intervals in doses of .5 c.c. In all, 20 c.c. were given with very little effect. Tetany distinct March 20 and 21.

	Mar. 18.	Mar. 19.	Mar. 20.							Mar. 21.									Mar. 22.		Mar. 23.	Mar. 24.
	3.00	4.30	2.30	3.45	5.00	7.00	8.00	9.00	11.00	1.00	3.00	5.00	7.00	9.00	11.00	1.00	3.00	5.00	9.00	12.00	2.00		5.30
KC..	0.1	0.1	0.05		0.05	0.05		0.05	0.05	0.2	0.1	0.2	0.2		0.2	0.1	0.1	0.1	0.05	0.3	0.2		0.05
KO..	1.4	1.4	0.1		0.1	0.2		0.2	0.3	0.6	0.4	0.4	0.5		0.5	0.4	0.2	0.1	0.3	0.4	1.1		0.3
AC..	0.6	0.6	0.1		0.1	0.2		0.1	0.1	0.3	0.2	0.3	0.2		0.1	0.2	0.1	0.1	0.1	0.4	0.5		0.3
AO..	0.7	0.5	0.2		0.2	0.2		0.3	0.3	0.5	0.3	0.3	0.4		0.2	0.2	0.2	0.2	0.3	0.6	0.5		0.4
				a			*a*				*a*					*a*		*a*					

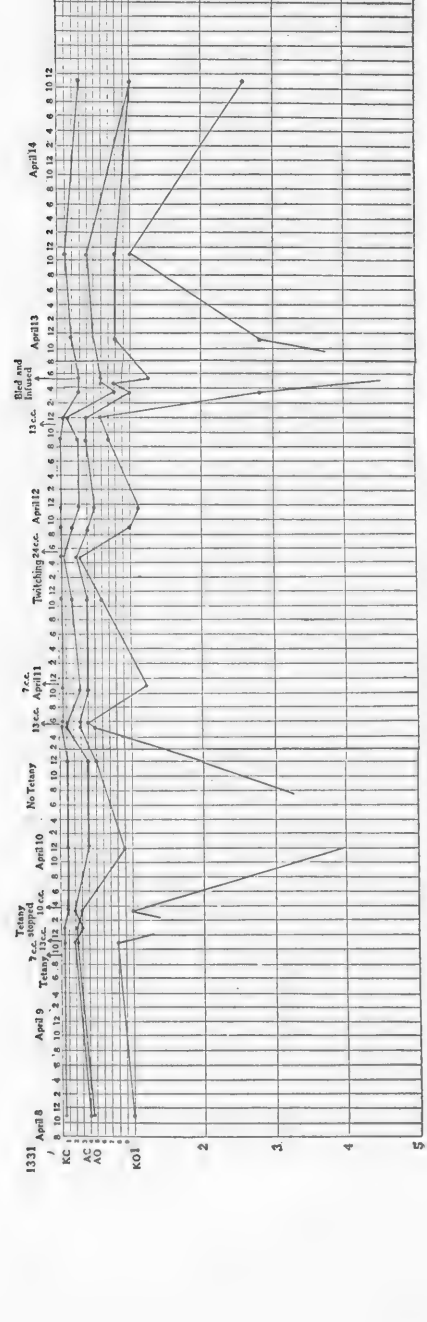
Parathyroid extract injected at *a*.

Larger doses were now used, as is shown in the following protocols.

Dog 1,331.—(Text-figure 7.) Parathyroids removed April 6. Violent tetany on April 9. Given doses of fresh extract from cow parathyroids ranging between 7 and 24 c.c. The extract was made with eighteen glands to 50 c.c. of Ringer's solution, and in such doses it seemed to have some effect on the excitability of the nerves. Even here, however, the effect is slight and of brief duration.

	Apr. 8.	Apr. 9.					Apr. 10.				Apr. 11.				Apr. 12.						Apr. 13.	Apr. 14.
	11.00	8.30	10.30	1.20	3.15	12.30	12 N.	5.20	5.50	10.30	11.30	4.30	8.15	11.40	9.45	12.00	3.25	4.45	5.10	11.50	¿11.00	11.00
KC..	0.05	Tetany	0.05	0.05	0.1	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.5	0.3	0.3	0.2	0.1	0.3
KO..	1.0		0.8	Negative at 6.0	1.0	Negative at 4.0	2.0	0.5	0.4	1.2	0.6	0.3	1.0	1.1	0.7	0.6	2.8	Negative at 4.0	Negative at 4.0	2.9	1.0	2.6
AC..	0.4		0.2	0.3	0.2	0.4	0.4	0.1	0.1	0.3	0.2	0.05	0.2	0.3	0.3	0.1	0.8	0.6	0.6	0.5	0.4	1.0
AO .	0.4		0.2	0.2	0.3	0.9	0.5	0.3	0.3	0.4	0.4	0.3	0.4	0.5	0.4	0.4	1.0	0.8	1.2	0.8	0.8	1.0
			a	*a*	*a*			*a*		*a*		*a*			*a*			*B*				

Parathyroid extract injected at *a*. At *B* 400 c.c. of blood were removed and 400 c.c. of normal blood injected.

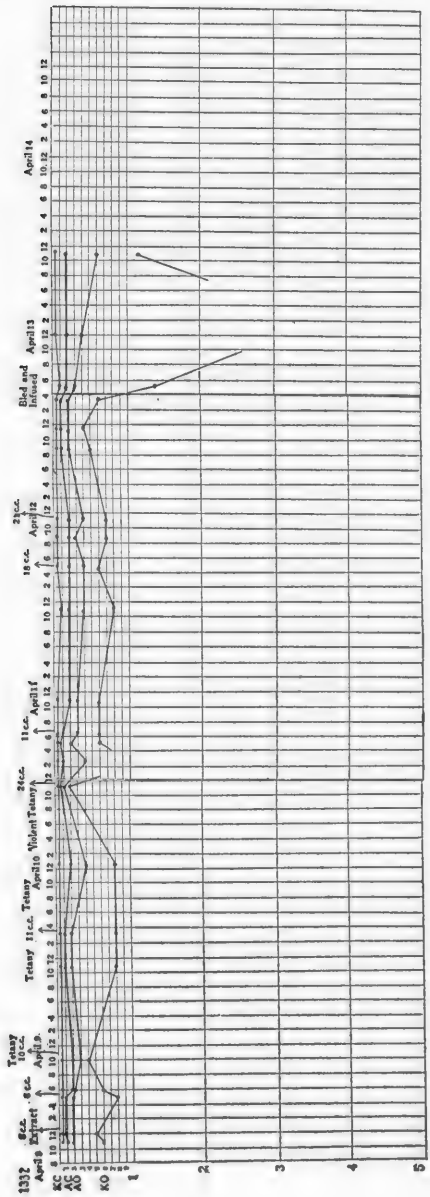


TEXT-FIG. 7. Tetany. Repeated injections of parathyroid extract. Finally bleeding with replacement by normal blood.

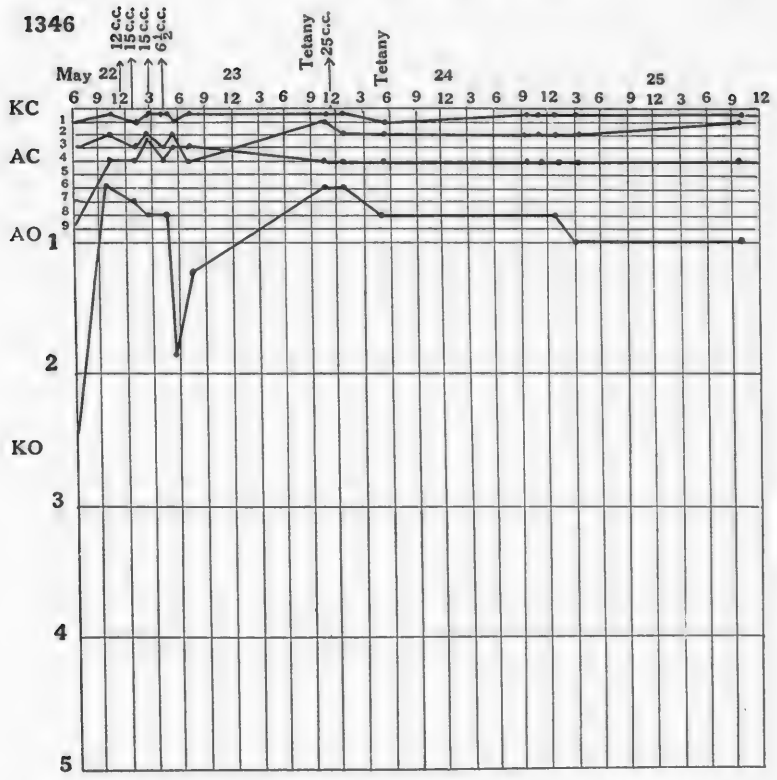
Dog 1,332.—(Text-figure 8.) Parathyroids removed April 6. Slight tetany began April 8. Given extract of dog's parathyroid, later of cow parathyroid; about twenty glands from the dog were injected. Extract from ox parathyroid made from eighteen glands in 50 c.c. of Ringer's solution. 12 to 24 c.c. were given at each dose. The effect in all cases was very slight, although the animal was kept alive nearly a week. On April 12 bled 400 c.c. which was replaced with 400 c.c. of normal blood.

	Apr. 8.					Apr. 9.			Apr. 10.					Apr. 11.				Apr. 12.						Apr. 13.
	11.00	12.00	5.00	6.00	10.00	11.00	3.30	12.35	11.30	2.45	5.20	6.10	10.30	11.20	4.20	8.30	11.30	9.50	11.55	3.25	4.30	5.00	12.00	11.00
KC...	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.05		0.1	0.05	0.05
KO...	0.6	0.5	0.8	0.6	0.4	0.8	0.8	0.8	0.2	Negative at 2.6	0.6	0.6	0.6	0.8	0.6	0.7	0.7	0.5	0.4	0.6		1.4	Negative at 4.0	1.2
AC...	0.1	0.05	0.05	0.2	0.2	0.1	0.1	0.2	0.05	0.1	0.05	0.01	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1		0.2	0.2	0.2
AO...	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.4	0.1	0.4	0.2	0.3	0.3	0.4	0.4	0.3	0.4	0.2	0.2	0.2		0.3	0.4	0.6
		a	*a*			*a*			*a*			*a*			*a*				*a*		*B*			

Parathyroid extract injected at *a*. Bled and transfused with normal blood at *B*.



TEXT-FIG. 8. Tetany. Repeated injections of parathyroid extract. Finally bleeding; replacement of blood with normal blood.



TEXT-FIG. 9. Tetany. Repeated injections of extract of dog's parathyroid.

It was feared that the slight effect of the injections of parathyroid extract might be due to the fact that ox parathyroids were used, and therefore an effort was made to obtain a sufficient number of the parathyroid glands of dogs to enable us to test the influence of an extract of those glands upon the tetany of a dog. In each instance about 100 glands were extracted in fifty cubic centimeters of Ringer's solution (without calcium.) In one case (dog 1,346) this fluid alone was used in extracting. In another (dog 1,350) glycerin was added. In neither instance did the effect seem greater than in the case of the cow parathyroid.

Dog 1,346.—(Text-figure 9.) Parathyroids removed May 19. Tetany on May 22. Large doses of extract were given at intervals, but the twitching persisted throughout the five days during which the dog lived.

	May 19.	May 20.	May 22.						May 23.			May 24.			May 25.		May 27.
			11.00	1.10	2.20	4.15	5.45	7.30	11.00	12.45	5.10	9.30	10.45	1.00	3.00	6.00	11.00
KC	0.2	0.1	0.05	0.1	0.05	0.05	0.1	0.05	0.05	0.05	0.1	0.05	0.05	0.05	0.05	0.05	0.08
KO	Negative at 5.0	Negative at 5.0	0.6	0.7	0.8	0.8	1.8	1.3	0.6	0.6	0.8	0.8	0.8	0.8	1.0	1.0	1.0
AC	0.5	0.5	0.2	0.3	0.2	0.3	0.2	0.4	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2
AO	2.0	1.0	0.4	0.4	0.2	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
				a	*a*	*a*	*a*			*a*							

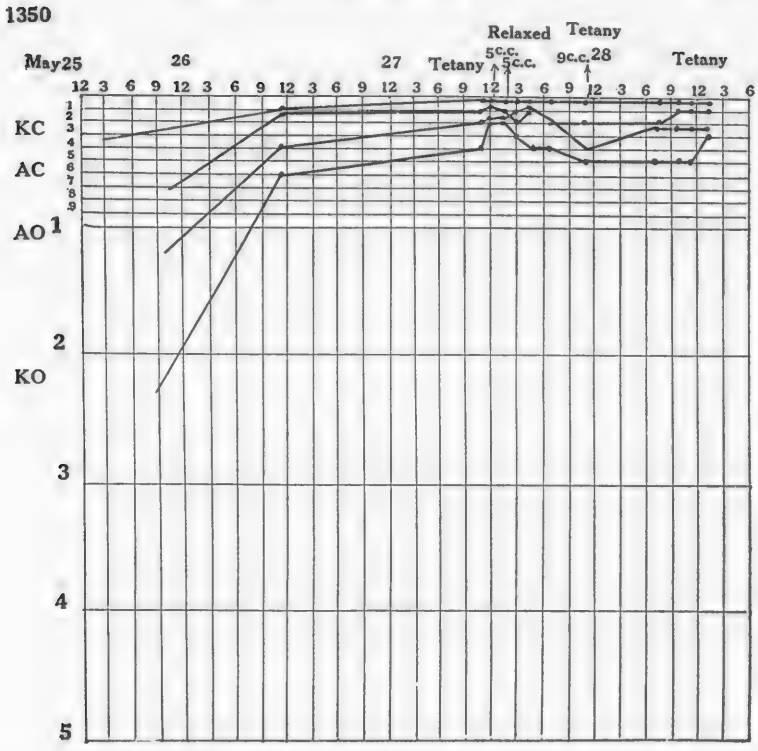
Parathyroid injections at *a*.

Dog 1,350.—(Text-figure 10.) Parathyroids removed May 25. Tetany varies and is sometimes outspoken; sometimes there is only a slight twitching in spite of the large doses of dog parathyroid.

	May 26.	May 27.							May 28.			
	11.30	11.00	12.00	2.00	3.00	4.30	6.15	11.00	8.20	9.30	11.00	1.00
KC..	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
KO..	0.6	0.4	0.2	0.2	0.3	0.4	0.4	0.5	0.6	0.5	0.5	0.3
AC ..	0.1	0.1	0.05	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.1
AO ..	0.4	0.2	0.2	0.2	0.1	0.1	0.2	0.4	0.2	0.2	0.2	0.2
				a	*a*			*a*				(Violent tetany)

Parathyroid injections at *a*.

The result of these experiments seems to show that even where relatively large doses of an extract of fresh parathyroid glands are administered in what would seem the most natural and most advantageous method (intravenously) to animals in tetany, the hyperexcitability of the nerves is not reduced at all or is lowered only temporarily and slightly, returning in a few hours to the original high level. Nor does it seem possible to keep an animal alive indefinitely by these injections when the parathyroid glands have been destroyed, although it is clear that life is distinctly prolonged



TEXT-FIG. 10. Tetany. Injection of parathyroid extract into vein.

thereby. One is disposed to ask whether there may not be some other feature of tetany apart from the hyperexcitability of the nerves which is beneficially affected by the extract.

The effect upon the hyperexcitability of the nerves is peculiar also inasmuch as the convulsions and twitchings practically disappear, and an animal found in complete rigidity with violent clonic contractions and tachypnea may be relieved and kept alive in a relaxed condition by the injection, although the electrical excitability of the peripheral nerves is practically unaltered. This is very different from the effect of injecting calcium under the same circumstances, for then the disappearance of the convulsions seems to be explained

by the extreme dampening of the excitability of the nerves. One can only imagine that in the case of the parathyroid injection the excitability may be lowered just below the threshold at which convulsions may occur. But the charts do not uphold this and there are many occasions when the electrical record is very high, though no twitchings are visible, while a few hours later with rather lower excitability there may be violent convulsions (text-figure 10).

Another possibility suggests itself, namely, that the excitability of the ganglion cells which send forth the impulses producing convulsions may be more readily affected by the parathyroid extract than that of the peripheral nerves. Such a condition would explain very well the rather curious effect of the extract. Even so the problem remains as to whether the death of the parathyroidectomized animal is the result of the mere mechanical exhaustion from the convulsions or of a general intoxication.

These experiments are unsatisfactory in throwing light on the question which we have set ourselves to decide,—whether the alteration in the blood in tetany is a mere loss of calcium, the presence of an oxalate-like substance capable of precipitating or rendering inactive the normal calcium, or the presence of a new poison somewhat like strychnia. The results might be compatible with the first view or even with the second if it proved that after neutralization of the oxalate-like substance the body failed to furnish enough fresh calcium to the tissues to make up the loss. Of course it may be that the extracts are faulty and inert in comparison with the activity of even the slightest amount of living parathyroid tissue. It may be that the gland acts not by way of any secretion but through its influence upon substances brought to its living cells by the circulating blood. And for that reason it may be that we are not really testing the third possibility with which these results seem so incompatible. A poison directly affecting the nerve cells might require living gland tissue for its destruction. Against this stands the fact brought out in the last paper that the blood of a normal animal will reduce the excitability of the nerves to normal after it has been heightened by tetany blood. Of course this in turn might be due to the presence of a sufficient amount of fresh calcium in the normal blood and the question is again unsettled. It might be

thought that the matter could be decided by bleeding an animal in tetany and restoring calcium-free Ringer's solution, and although this has been done and results as a matter of fact in the lowering of the excitability, it does so also in normal animals and therefore is not specific. Possibly it is due to a disturbance of the nutrition of the nerves. A few protocols will illustrate this point.

Dog 1,321.—(Text-figure 11.) Normal animal with average excitability. Bled 325 c.c. Blood replaced by 500 c.c. of calcium-free Ringer's solution. Excitability tested before and each hour after this operation.

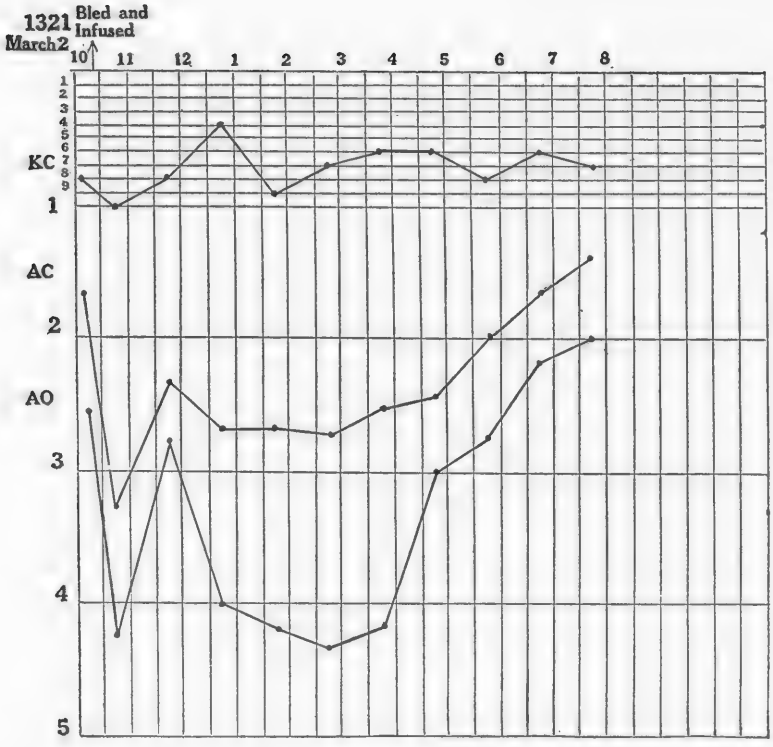
	Mar. 2.												Mar. 3.
	10.10	10.15	10.45	11.45	12.45	1.45	2.45	3.45	4.45	5.45	6.45	7.45	
KC ...	0.8	Bled and infused.	1.0	0.8	0.4	0.9	0.7	0.6	0.6	0.8	0.6	0.7	0.4
KO ...	Negative at 5.0		—	—	—	—	—	—	—	—	—	—	—
AC....	1.6		3.2	2.4	2.6	2.6	2.7	2.5	2.4	2.0	1.7	1.4	1.2
AO....	2.6		4.2	2.8	4.0	4.2	4.4	4.2	3.0	2.8	2.2	2.0	3.4

Dog 1,324.—Large normal dog; weight 30 kilos. Bled 630 c.c. from carotid. 1,400 c.c. of Ringer's solution without calcium were run into the jugular vein.

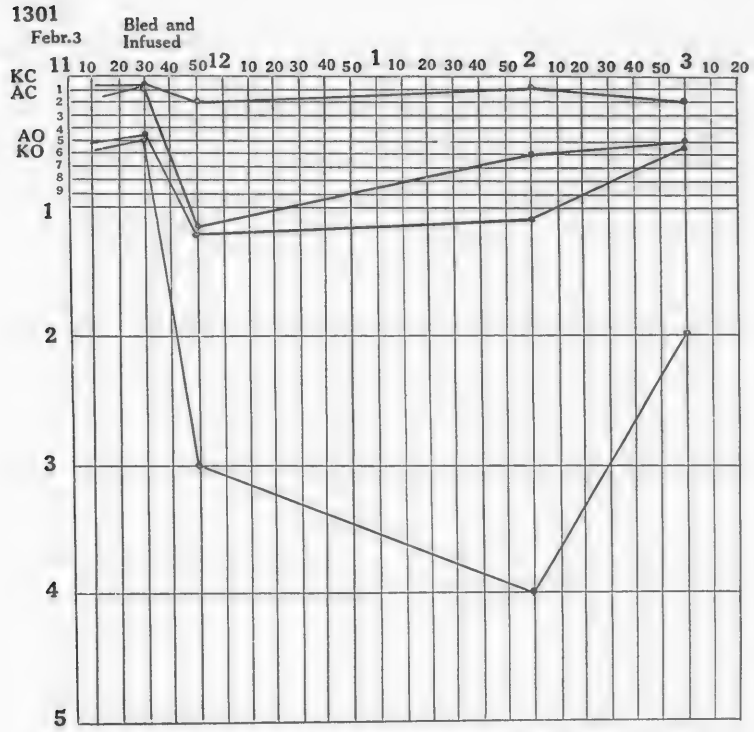
	Mar. 3.				
	12.00	12.15	12.30	2.00	5.30
KC.....	0.3	Bled and infused.	0.3	0.3	0.3
KO.....	Negative at 5		——	——	——
AC.....	0.6		1.0	1.6	1.9
AO.....	1.2		2.4	2.8	3.0

Dog 1,301.—(Text-figure 12.) Parathyroids removed January 31. Violent tetany February 3, 11.30 A. M. Bled 450 c.c. 250 c.c. of Ringer's solution without calcium were injected. Died at 5 P. M.

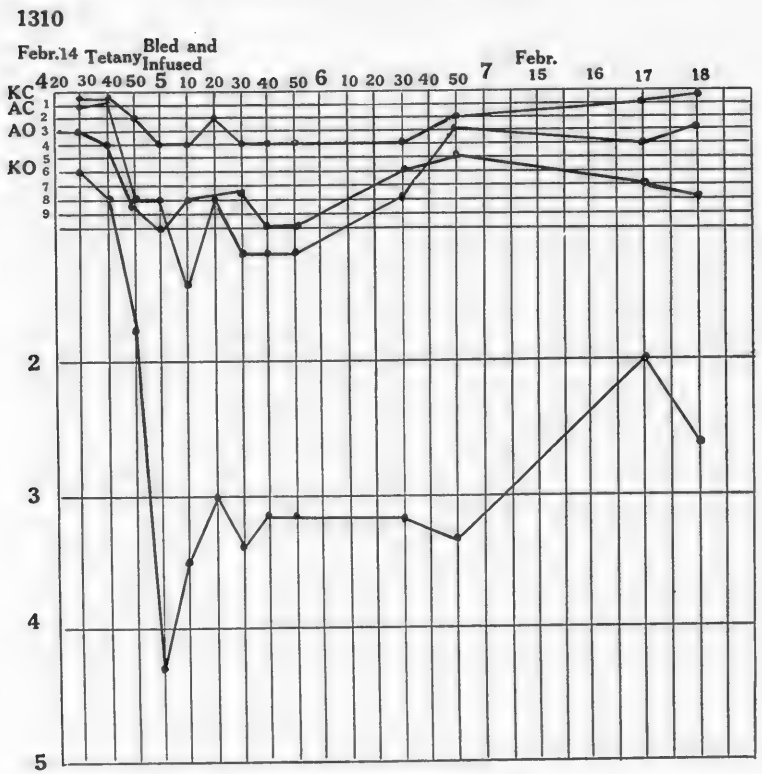
	Feb. 3.				
	11.30	11.40	11.45	2.00	3.00
KC.....	0.05	Bled and infused.	0.2	0.1	0.2
KO.....	0.5		3.0	4.0	2.0
AC.....	0.05		1.2	0.6	0.5
AO.....	0.5		1.2	1.1	0.5



TEXT-FIG. 11. Bleeding followed by injection of Ringer's solution without calcium.



TEXT-FIG. 12. Tetany. Bled 450 c.c.; 250 c.c. Ringer's solution without calcium returned.



TEXT-FIG. 13. Tetany. Bled 500 c.c.; 1,200 c.c. Ringer's solution without calcium in vein.

Dog 1,310.—(Text-figure 13.) Parathyroids removed February 9. Tetany distinct February 14, 4 P. M. Bled 500 c.c. at 4.50 P. M. 1,200 c.c. of Ringer's solution without calcium were injected into the jugular at 5.10 P. M.

	Feb. 14.													Feb. 15.	Feb. 16.	Feb. 17.	Feb. 18.
	4.30	4.40	4.50	5.00	5.10	5.20	5.30	5.40	5.50	6.00	6.10	6.20	6.30	6.50				
KC.....	0.05	0.05	0.2	0.4	0.4	0.2	0.4	0.4	0.4				0.4	0.2			0.1	0.05
KO.....	0.6	0.8	1.8	4.4	3.6	3.0	3.4	3.2	3.2				3.2	3.4			2.0	2.6
AC.....	0.1	0.5	0.8	0.8	1.4	0.8	1.2	1.2	1.2				0.8	0.3			0.4	0.3
AO.....	0.3	0.4	0.8	1.0	0.8		0.8	1.0	1.0				0.6	0.5			0.7	0.8
		Bled			Infused													

Dog 1,314.—(Text-figure 14.) Parathyroids removed February 18. High excitability but not violent tetany on February 21. At 3.40 bled 500 c.c. 1,000 c.c. of Ringer's solution without calcium were introduced.

	Feb. 20.	Feb. 21.					Feb. 22.
		3.30	3.50	4.45	5.45	7.45	11.15
KC.....	0.1	0.05	0.4	0.4	0.4	0.2	0.05
KO.....	Negative at 3.0	0.6	3.0	2.0	Negative at 3.0	4.0	2.0
AC.....	0.2	0.1	1.2	0.6	0.8	0.8	0.4
AO.....	0.4	0.3	1.0	1.0	1.4	1.4	0.6

One feels almost sure from these experiments that the method is too coarse to throw any light upon the nature of the alteration of the blood in tetany. The excitability of the nerves, as we have seen from perfusion experiments and those concerned with anemia, is a property so delicate that it is readily disturbed or destroyed by measures which change in any marked degree the character or amount of the nutritive blood supply. It is probably for this reason that the symptoms of tetany may be relieved, as Meltzer[2] pointed out, by the injection of a great amount of strong salt solution, and not because of any specific action of the sodium chloride. That such effects pass off after a time is due doubtless to the power of the body to reëstablish the normal composition of the blood.

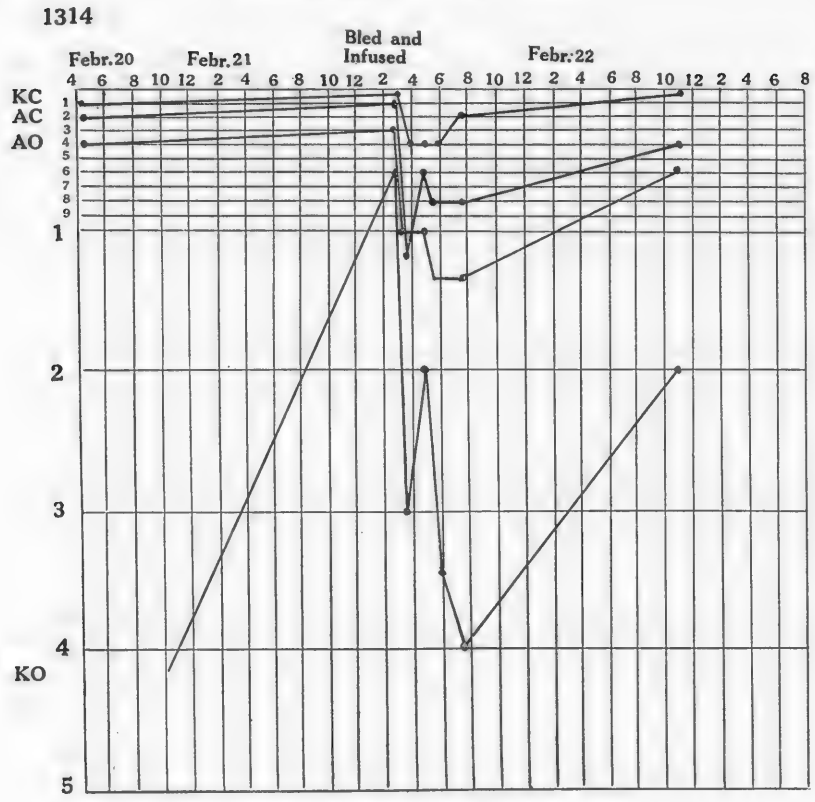
Other methods of attack which we have previously used have been employed again, and the results will be recorded here.

Since we know definitely that the blood of an animal in tetany differs from that of a normal animal, it would at first sight seem possible to study the blood directly, but the difficulties are very great. Direct analyses to show the calcium content can of course be made, and some have been recorded in a previous paper,[3] showing a marked decrease in the calcium content during tetany.

It was thought that chemical analyses might contribute something to the understanding of the action of the parathyroid extract, if we should determine any differences between the calcium contents of the blood of untreated dogs suffering from tetany and that of dogs

[2] Joseph, D. R., and Meltzer, S. J., *Jour. Pharmacol. and Exper. Therap.*, 1910–11, ii, 361. Voegtlin, C., and MacCallum, W. G., *ibid.*, p. 439.

[3] MacCallum, W. G., and Voegtlin, C., *Jour. Exper. Med.*, 1909, xi, 142.



TEXT-FIG. 14. Tetany? At 3 P. M. on February 21 bled 500 c.c., 1,000 c.c. of Ringer's solution returned.

which had received repeated doses of parathyroid extract. In other cases we analyzed the blood of dogs from which most of the parathyroid tissue had been removed because, as we supposed, some of the gland tissue had remained uninjured. There are therefore four categories into which these animals fall.

Normal.	Tetany.	Tetany treated with parathyroid extract.	Partial parathyroidectomy without tetany.
Dog 1,324—0.0624	Dog 1,349—0.0156	Dog 1,350—0.0200	Dog 1,344—0.0541
Dog 1,360—0.0731	Dog 1,352—0.0322	Dog 1,331—0.0264	Dog 1,345—0.0444
Dog 1,361—0.0580	Dog 1,348—0.0226	Dog 1,332—0.0436	
Dog 1,362—0.0502	Dog 1,353—0.0390		

These figures represent the content in calcium in grams of 1,000 grams of blood. The analyses were made by the method of Neumann, the calcium being estimated gravimetrically and not by titration with potassium permanganate. They were made without knowledge of the history of each case and compared only after all were finished. It is plain from these figures that the calcium content of the blood of normal dogs and of those which did not develop any symptoms of tetany even though the parathyroid gland tissue was much reduced is much higher than in the other two groups. It may be observed further that the great decrease in the amount of calcium in the blood of the animals which show tetany persists in spite of the injection of parathyroid extract, a point which is significant and in accord with the results of the electrical tests of nervous excitability. Probably we may conclude from this that the idea that parathyroid extract might stop the tetany by heightening in some way the calcium content of the blood is without actual foundation.

Several attempts were made in the direction of imitating the action of a possible oxalate-like substance in the blood by injecting a solution of sodium oxalate into the vein. In a previous paper it was shown that the addition of a minute quantity of sodium oxalate to the normal blood used in perfusing a limb caused a hyperexcitability of the nerves, but that it was difficult to carry out this experiment without killing the nerves. Sarvonat and Roubier[4] have shown that the prolonged administration of oxalates by mouth will finally produce such hyperexcitability which could be measured electrically. The injection of quite large quantities of the oxalate into the veins of an animal should produce such a change, but several experiments of this sort have given practically no result although the animal sometimes died during the injection. The solution was then allowed to run into the vein much more slowly, and although it caused vomiting and depression it produced no especial change in the excitability of the nerves. When, however, the same animal was again injected the next day or the day after, the effect became noticeable (text-figure 15).

[4] Sarvonat, F., and Roubier, Ch., *Arch. de méd. expér.*, 1911, xxiii, 584; *Jour. de physiol. et de path. gén.*, 1911, xiii, 565.

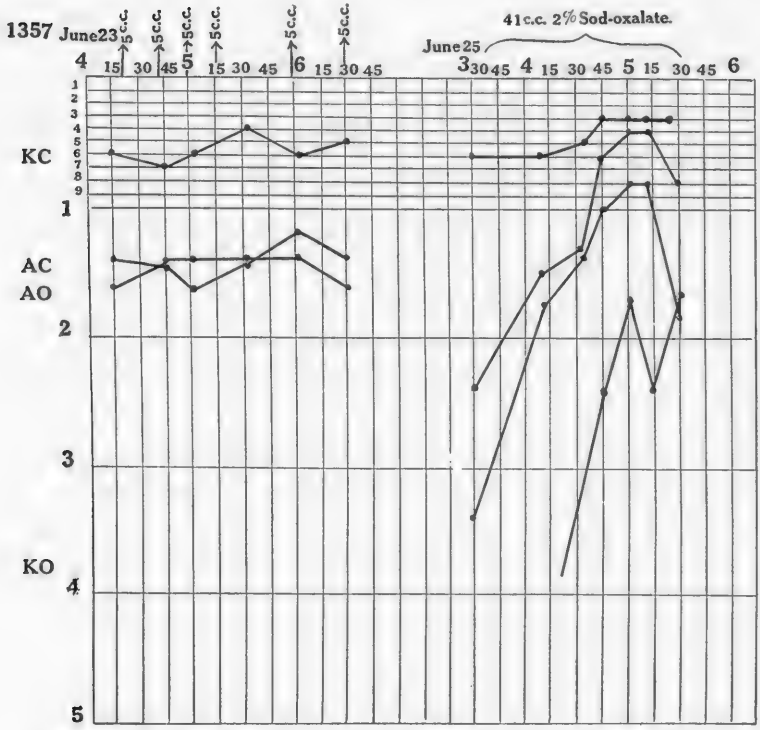
Dog 1,357.—(Text-figure 15.) Normal animal. On June 23 a 2 per cent. solution of sodium oxalate was slowly run into the vein so that several minutes were occupied in the introduction of 5 c.c. No especial effect was produced. 30 c.c. of the solution were administered. Two days later 41 c.c. of the same solution were run into the vein continuously but very slowly. This produced a distinct rise in the excitability of the nerves.

	June 23.						June 25.						
	4.15	4.45	5.05	5.35	6.05	6.30	3.30	4.10	4.35	4.45	5.00	5.10	5.30
KC.....	0.6	0.7	0.6	0.4	0.6	0.5	0.6	0.6	0.5	0.3	0.3	0.3	0.3
KO.....	Negative at 5.0	—	—	—	—	—	Negative at 5.0	Negative at 5.0	Negative at 5.0	2.4	1.8	2.4	1.8
AC.....	1.6	1.4	1.4	1.4	1.4	1.6	3.4	1.8	1.4	1.0	0.8	0.8	1.8
AO.....	1.4	1.4	1.6	1.4	1.2	1.4	2.4	1.6	1.4	0.6	0.4	0.4	0.8
		30 c.c. 2 per cent. oxalate.						41 c.c. 2 per cent. oxalate.					

It is important that more experiments lasting over a longer period should be carried out, since the body seems to be able to repair for a time the injury to the blood caused by the oxalate. It was quite remarkable to observe that the heart blood taken from a small dog which had died during the administration of the 2 per cent. oxalate after twenty-eight centimeters had run in, clotted firmly within five minutes. It seemed that this amount should be sufficient to prevent the clotting in a small dog weighing only about five kilograms, but apparently the circulating blood can protect itself. When the introduction of oxalate is repeated this protection seems to become less and less available, until finally the effect is observed in the nerves.

SUMMARY.

It has been our aim to determine in what particular the blood is altered during tetany so that it can produce hyperexcitability of the motor nerves. As a working hypothesis it has been assumed that (1) there may be a lack of calcium in the blood and tissues; (2) there may be a circulating poison which like an oxalate could render inactive the circulating calcium; and (3) there may be a substance in circulation vaguely resembling strychnia in its action upon the nervous system and directly causing the hyperexcitability. It is



TEXT-FIG. 15. Injection of 2 per cent. sodium oxalate into jugular vein.

impossible to decide with our present knowledge which, if any, of these explanations is the true one, but certain experimental results are brought forward. If tetany blood be used to perfuse a normal leg the excitability of the nerves rises to a characteristically high level and the addition of parathyroid extract to the blood has little or no effect in lowering this excitability.

Parathyroid extract whether from the ox or the dog fails when injected into the circulation of an animal in tetany to reduce the excitability of the nerves markedly or permanently, although it seems to affect the more sensitive ganglion cells thus cutting off excessive impulses to the periphery. Although the nerves remain hyperexcitable, tetany is usually much diminished or abolished

entirely. This seems to be analogous to the action of ether or any other anesthetic which may inhibit the activities of the ganglion cells, although it leaves the nerves excitable and able to conduct impulses. An animal in tetany relaxes instantly on being given ether although the excitability of the nerves to electric currents is little changed.

Bleeding followed by the replacement of the blood with an indifferent solution free from calcium stops tetany and lowers the excitability of the nerves. Probably this cannot be ascribed to the removal of a circulating poison, but rather to a general disturbance of the nutrition of the nervous system.

Oxalate-like substances introduced into the circulation rapidly, and for a short time only, may kill the animal, but they seem to produce no change in the excitability of the nerves. If the solution is injected very slowly and over a long period the protective action of the body seems to be overcome and the excitability of the nerves rises to high levels. This seems to resemble somewhat the latent period after the destruction of the parathyroid before tetany begins.

Direct analysis of the blood shows that as compared with the normal, the blood of an animal in tetany is very poor in calcium. Administration of parathyroid extract does not increase this calcium content. On the contrary, if the extirpation of the parathyroids has been incomplete so that tetany does not appear, the calcium content of the blood is that of the normal animal.

Even yet, therefore, in spite of our efforts to shake it, the theory that tetany is closely dependent upon a disturbance of the calcium content of the blood is supported by stronger evidence than any other idea, but much remains to be done before a clear conception of the process is reached.

THE CHARACTERS OF A THIRD TRANSPLANTABLE CHICKEN TUMOR DUE TO A FILTERABLE CAUSE. A SARCOMA OF INTRACANALICULAR PATTERN.*

By PEYTON ROUS, M.D., and LINDA B. LANGE, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

Plates 49 to 55.

That tumors of the fowl are far from rare is now recognized; and there exists a considerable literature concerning them.[1] Efforts to transplant the growths have met in general with poor success. Thus far only three transplantable chicken tumors have been described. Three others are at present under propagation in our laboratory, one of which forms the subject of the present paper. This tumor owes its transplantation to the use of many fowls as hosts. Probably the failure to transfer chicken tumors has often been due to neglect of the point. If only two or three animals were used in each attempt to transfer spontaneous mouse tumors the results would certainly be unsatisfactory.[2]

The three fowl tumors previously described are a spindle-celled sarcoma,[3] a myxosarcoma,[4] and an osteochondrosarcoma.[5] The first and last of these growths have been reported upon in detail.

* Received for publication, July 3, 1913.

[1] Ehrenreich and Michaelis, Tyzzer, Rous, Murphy, Wernicke, Tytler.

[2] Some tumors of the fowl, like some tumors of the mouse, resist all attempts at transplantation. The epitheliomata of the scaly portion of the leg are among these. During the past two years five such spontaneous epitheliomata have been brought to the laboratory; and more than one hundred young adult chickens have been inoculated from them, often in several ways, but without success.

[3] Rous, P., *Jour. Exper. Med.*, 1910, xii, 696.

[4] Fujinimi, A., and Inamato, K., *Verhandl. d. Jap. path. Gesellsch.*, 1911, 114.

[5] Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1682; Tytler, W. H., *Jour. Exper. Med.*, 1913, xvii, 466.

They have interest not only as characteristic neoplasms in the bird, but because the cause of each has been found in an agent separable from the tumor tissue by filtration through a Berkefeld filter.[6] The growth now to be described is quite as characteristically a neoplasm and also has a filterable cause. The experiments upon its etiology will be published in a separate paper. It is a connective tissue tumor, a sarcoma split and fissured by many compressed blood sinuses into which it tends to extend and grow in an intracanalicular pattern. Its like has not been found among the forty-three spontaneous chicken tumors recently studied by us.

THE ORIGINAL GROWTH.

The fowl with the spontaneous tumor was brought to us because of swellings in the left leg that rendered it lame. The swellings proved to be two smooth and very firm masses, lying in the muscle above and below the knee joint. They seemed attached to the bone and limited the movement at the joint in both directions. The fowl, a mongrel, brown Leghorn hen, was sparely nourished; it died under chloroform during an exploratory operation. Inoculation of portions of the tumors were made within three hours into ten Leghorn chickens.

Gross Findings.—In the gizzard was a mass which may with good reason be regarded as the primary growth. It occupied nearly the whole right anterior portion of the organ, and projected irregularly under a covering of mesentery (figure 1) in which lay several small nodules up to 0.6 cm. in diameter, some sessile on the gizzard, some free. On section the mass proved roughly spherical, measuring 4.3 by 4.3 cm. (figure 2). It was everywhere surrounded by muscle and though it encroached on the gizzard cavity the mucous membrane was not eroded. It had no capsule but was sharply demarcated by its color, a pale, pinkish white, from the wine-colored muscle. It bulged on the cut surface and was made up of many irregular, tightly compressed subdivisions separated by minute, irregular fissures. Toward one side was a localized soft, yellow necrosis, but the growth was in general translucent and, though poorly vascularized, appeared sound. In the fold of mesentery extending from the gizzard to the left lobe of the liver was a flattened grayish pink nodule measuring 0.2 by 0.05 cm.

The liver was enlarged, congested, mottled with ill defined, pale areas. On its anterior surface were two small, stellate depressions marking irregular, gray areas in the parenchyma that suggested scars. These areas were later found to consist of tumor tissue. The other viscera appeared normal.

In the substance of the extensor muscles of the left thigh was an oblong, smooth mass 4.5 by 3 by 3 cm. with its long diameter in the direction of the muscle. A similar mass 4 by 3 by 3 cm. occupied the muscles just below the knee (figure 3). The upper mass was firmly attached to the periosteum for

[6] Rous, P., *Jour. Am. Med. Assn.*, 1911, lvi, 198; *Jour. Exper. Med., loc. cit.;* Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lix, 1793.

about 2 cm. above the patella, and to the joint capsule also. A prolongation extended from it around to the back of the joint. The lower mass was attached to the joint capsule but the bulk of it lay in the muscle. The growths were extremely firm, much firmer than the one in the gizzard, pinkish white, nearly bloodless, and consisted of finely striated tissue, in part solid and in part, like the tumor of the gizzard, divided by fissures into irregular lobuli. Some of the lobuli had a central, semigelatinous depression, and in a few the center was yellow, firm, and opaque,—evidently necrotic. The growths were unencapsulated and poorly defined from the muscle sheaths, periosteum, and tendons which they involved.

The knee joint was full of papillary proliferations (figure 3), translucent and gristly, attached to the capsule, or joint surface. The largest one had its base on the posterior surface of the patella. Microscopically all were found to be extensions from the growths on the outer surface of the capsule. Within the lower end of the femur, lying in the red marrow, was a translucent tumor mass about 0.4 cm. in diameter. The bone from without had seemed normal.

In the muscle of the chest wall just anterior to the junction of the sternal and vertebral portions of the three upper right ribs was a tumor measuring 2.5 by 1.4 by 1 cm., which projected into the thoracic cavity (figure 1). In the anterior muscles of the neck, just above the left clavicle, was a similar mass, 3.9 by 1.7 by 1.5 cm. in size; and yet a third, measuring 3 by 2 by 1 cm., lay in the muscle of the inner surface of the pelvis near the right anterior portion of its rim. All resembled the growths in the leg.

Microscopic Findings.—The tumor of the gizzard consists of a spindle-celled tissue irregularly split and separated into islands by many fairly wide channels now quite empty except for a few blood cells (figure 4). The channels are walled by only a single layer of endothelium and some of the smaller ones lack even this and are probably artefacts due to shrinkage in the fixing fluid. The prominence of the larger channels is doubtless dependent to some extent on the same cause, since they are nearly empty and the tumor in the gross was practically bloodless.

Mitoses are fairly frequent. The tumor's enlargement has obviously taken place by intrinsic proliferation coupled with the direct invasion and replacement of normal structures. In the muscle just beyond the growth's border, and not yet attacked, are numerous dilated blood spaces and capillaries. Within the tumor the sinuses thus formed have become more extensive but are filled for the most part with papilliferous extensions of tumor tissue covered everywhere by endothelium. Many of the sinuses are compressed to mere slits.

The metastases in the muscles (figure 5) closely resemble in the main the growth in the gizzard. In some parts, however, sinuses are infrequent and the tissue is practically solid.

Briefly, the curious tumor,—called in this laboratory Chicken Tumor XVIII,—consists of a spindle-celled, sarcomatous tissue fissured and subdivided by many flattened sinuses, and often intracanalicular in its growth (figures 4 and 5). The primary mass, as

the later findings have shown, lay in the wall of the gizzard (figures 1 and 2). Secondary growths were present at several points in the skeletal muscles, notably in the left leg where were two large masses, one of which had extended into the knee joint and the marrow of the femur (figure 3).

TRANSPLANTATIONS.

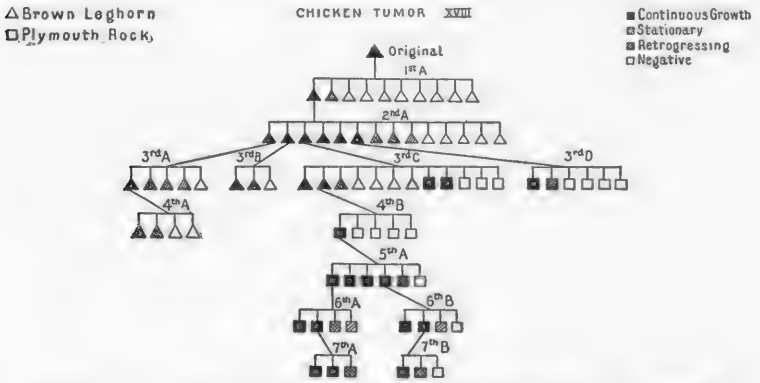
Transplantations of the tumor were made three hours after the host's death, by the insertion through a trocar of small bits of the fresh tissue deep in the pectoral muscle of normal fowls. The tissue used was taken from one of the growths in the left leg. The fowls were young brown Leghorns, chosen for their similarity to the original fowl, since previous observations had shown[7] that the success of transplantation sometimes depends on the use of chickens of the same strain as the original. The fowls received two implantations, one in each breast.

Growths developed in only two of the chickens and in one of these eventually retrogressed. The nodules became palpable within a month, but their enlargement was extremely slow. That they were the expression of a general susceptibility is shown by their development at both sites of inoculation. Material for the second tumor generation was obtained by removing, forty-six days after inoculation, the tumor largest at this time. A recurrence promptly took place at the site of operation but its growth was slow. Both fowls were alive and active eight months after inoculation, one with growths still retrogressing from a maximum diameter of two centimeters, the other with masses that had gradually increased to five centimeters across. Both were now inoculated in the leg muscles with another and more malignant chicken tumor (Chicken Tumor I, a spindle-celled sarcoma) and of this they eventually died, though not till one had rounded out nearly a year as tumor host.

Chicken Tumor XVIII is now in its eighth tumor generation. It has been pushed thus far in fifteen months by transplanting from the sarcomatous nodules shortly after their appearance. With repeated transfer the percentage of successful inoculations and the rate of growth have increased markedly (text-figure 1). In the earlier generations care was taken to select hosts of brown Leghorn

[7] Rous, P., *Jour. Exper. Med., loc. cit.*

strain. Though the percentage of takes increased, the growth of the tumor was still slow when, for the third generation, series C, five brown Leghorn and six Plymouth Rock fowls were inoculated. In two hosts of each sort tumors developed, and in one Plymouth Rock they grew with unexampled rapidity. Retransplantation to other



TEXT-FIG. 1. Results of the transplantation of Chicken Tumor XVIII into seven consecutive series of fowls. The percentage of successful inoculations has been much increased, and seems little influenced by the variety of the host.

Plymouth Rock fowls resulted in a still further increase in rapidity of growth and some changes in structure of the tumor and distribution of metastases. In the absence of direct comparative tests it cannot be said that the findings in Plymouth Rocks represent a special susceptibility of these fowls, because similar results might have followed a fortunate transplantation in brown Leghorns. But the facts show that Chicken Tumor XVIII, like Chicken Tumor VII (an osteochondrosarcoma), and unlike Chicken Tumor I (a spindle-celled sarcoma) in its earlier generations, can be readily transplanted to fowls of another variety than that in which it occurred spontaneously.

BEHAVIOR OF THE TRANSPLANTED TUMORS.

The usual history of one of the tumors of the early generations in Leghorn fowls is as follows:

Two or three weeks after the implantation a small, firm nodule

appears where the needle has been thrust deep in the breast. This slowly enlarges with its long axis in the direction taken by the muscle fibers. On section it consists of an extremely firm, bulging, finely striated, grayish pink tissue, nearly bloodless, and in part finely subdivided by minute fissures. Small areas of necrosis or hemorrhage may be present. There is no capsule. Enlargement of the nodule takes place so slowly that only after from four to seven months does it attain a diameter of five centimeters. It is still extremely firm and somewhat elastic, on palpation suggesting cartilage, and is sound practically throughout. The host though still active emaciates as the mass becomes large and the wasting of the pectorals throws the tumor into relief. Its surface is smooth and it is irregularly football-shaped or shaped like a flattened sphere. The cut surface may be finely fissured, but there is little indication of the elaborate, intracanalicular arrangement visible macroscopically. Rarely the skin over the tumor becomes attached, or the growth fixes itself to the sternum. In resistant hosts the mass is encapsulated and may show a gross lobulation.

When the tumor has become large, secondary growths appear in the limbs of some hosts. The fowl limps and its wings seem stiff. On examination hard, elastic masses are found lying as a rule in the muscle above or below the knee joint anterior to the bone, and near the first joint of the wing on its under surface. They sometimes occur in the muscles of the neck, and in the intercostals, and about the hip. There are often several but never many of the secondary growths in one fowl, and each seems to arise by enlargement from a solitary focus. They start near a joint as a rule, sometimes from the outer surface of its capsule, and extend along and within the muscles and tendons as discrete, smooth, firm masses of much larger bulk than the normal tissues which they replace. They hinder movement greatly, and by binding the muscles to the periosteum may practically prevent it. The points at which they appear in the wing and leg are those much exposed to trauma.

The fowl in which secondary growths have developed presents a truly remarkable picture (figure 6). The wings, held close, in nearly the normal position, are not paralyzed but are stiffened,

sometimes to immovability, by firm masses lying in the muscle substance. The legs are greatly swollen about the knee and are nearly immovable at this joint. To either side of the sternum are the large, projecting, primary growths. With all this the fowl may be vigorous; and the growths give it a factitious plumpness. But sooner or later it goes into coma and a day or two later dies.

The viscera are usually free from metastases. A few small nodules may be present in the lungs. In one instance they were found in the heart. In a fowl of the second tumor generation, dying between five and six months after inoculation, there were, in addition to metastases in the wings and legs, several localizations in the skin of the body, appearing as plateau-like placques, or thickenings, raised three or four millimeters above the surface. These placques were roughly oval, circumscribed, and consisted of tumor tissue growing in the loose, superficial layer of the corium and limited by its dense, sheet-like, deep layer. The epidermis over them was not ulcerated or discolored, but the pitting of the feather follicles was much exaggerated. The viscera in this case were free of tumor.

At every autopsy the gizzard has been carefully examined. Secondary growths have not been found in it. That the tumor in the gizzard of the original fowl was primary and those in the muscles metastases seems certain.

In the Plymouth Rock fowls of the fourth to eighth tumor generations the tumor sometimes grew so rapidly as to measure five centimeters across one month after the inoculation, shortly occupying the entire breast. It has retained its firm, dense character, but no longer gives metastases to the limbs. Metastases have been found several times in the lungs, less often in the heart and liver, and once in the omentum; but in general secondary growths are rare. Those in the lungs may coalesce to render the organ semisolid.

HISTOLOGY.

In the earlier generations (brown Leghorn fowls) the tumor retained the structure of the spontaneous growth. The fissuring was often very marked and intracanalicular extension not infrequent (figure 7). Both features were found in primary and metastatic

growths, situated in the voluntary muscles; but in growths of the lungs, heart, and skin they were absent, and the tissue was that of a pure, spindle-celled sarcoma (figure 8). The structure of the organ involved, and the peculiar fashion of the growth's invasion, as interacting factors, are doubtless responsible for this difference. In its method of invasion and replacement, as exemplified in voluntary muscle, Chicken Tumor XVIII differs from other chicken tumors thus far studied. Leaving out of consideration the method of pressure atrophy common to all, Chicken Tumor I, a spindle-celled sarcoma, destroys the muscle, either by a direct apposition of tumor cells to its fibers, associated with what may be called erosion, or by a direct extension into the interior of the muscle fibers and proliferation there. Figures illustrating this have been published.[8] Chicken Tumor VII, the osteochondrosarcoma, replaces the muscle in both ways, doing so usually by the first. But Chicken Tumor XVIII, by means of strands of proliferating cells, splits up each muscle fiber longitudinally into a number of fragments which are then absorbed (figure 9). In cross-sections of the muscle at a certain period in the process of replacement a hundred isolated muscle bits, each apparently an atrophying unit, lie in the space normally occupied by twenty or thirty muscle fibers. They are derived indeed only from this number. During the replacement the preëxisting blood spaces enlarge; intracanalicular growth into them takes place to greater or less extent; and there results a tissue irregularly subdivided by blood sinuses, and often to a large extent lying within them. The sinuses are always greatly compressed and nearly empty of cells. Many of the smaller clefts are not lined by endothelium, and are presumably artefacts due to shrinkage.

The extension of the tumor by invasion and replacement is more obvious than by expansion. Bone may be rapidly eroded. The firmness of the tissue is traceable to the large quantity of collagen distributed in it, often as thick, undulating ribbons or bundles. Next to these bundles the cells may be grouped regularly, in strata, but generally they lie in irregular strands or whorls, and the collagen fibers are irregularly distributed. In the more rapidly growing areas mitotic figures are frequent. There is a noteworthy

[8] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1913, xvii, 219.

absence of giant cells. The cells have considerable uniformity but vary somewhat in size. The nucleus is large and vesicular, the cytoplasm scanty, and the cell outline that of a short spindle. More or less attenuated cells are frequent, and occasionally very slender ones are seen, grouped in strands, with rod-shaped nuclei, suggesting to some extent smooth muscle. Focal hydropic changes occur (figure 10) and rarely a mucinous degeneration.

In the later generations of the tumor, those of its rapid growth in Plymouth Rock fowls, it is usually a pure, spindle-celled sarcoma, fissured by a few flattened sinuses. It has become more cellular, though collagen fibrils still render it firm. Degenerative forms are still absent.

In very susceptible fowls no cellular reaction is found at the tumor's border except that associated with its support and vascularization. Always there is a total absence of histological features suggesting infection. In fowls relatively resistant, as shown by the tumor's slow growth, bands of scar tissue divide it irregularly, and small round cells, with some macrophages, are massed at its border and scattered within it. The neoplastic tissue disappears, not by necrosis *en masse,* but by a gradual absorption which is compatible with survival of the cells for a long time. The histological phenomena are quite similar to those associated with the retrogression of mammalian tumors[9] and have been described at length in connection with another neoplasm of the fowl.[10]

RESISTANT HOSTS.

The tumor has not grown in pigeons, rats, or mice. Ten individuals of each sort were inoculated with material that was active, as shown by the results in chickens. The tumor fails to grow in some chickens of the susceptible varieties, and in others, after a greater or less development, it retrogresses. The question whether retrogression results in a heightened resistance, has not been settled. Fowls in which the tumor is growing can be successfully reinoculated.

[9] Da Fano, C., *Ztschr. f. Immunitätsforsch., Orig.,* 1910, v, 1.
[10] Rous, P., and Murphy, J. B., *Jour. Exper. Med.,* 1912, xv, 270.

TRANSPLANTABILITY.

In studying the transplantability of the tumor the fate of bits of it implanted intradermally has been followed. In some fowls a suspension of the tumor tissue has been injected intravenously and the lungs examined later in serial section, but these inoculations, so successful with Chicken Tumor I, have not resulted in tumors. Similar negative results from intravenous inoculation are recorded of several neoplasms of the rat and mouse.[11] The intradermal method has proven very satisfactory. Eight to ten tumor bits are implanted in each of a number of fowls, and are removed on successive days with the tissue about them, and submitted to serial section. In susceptible hosts the implanted cells survive, are vascularized, proliferate, and in a short time give rise to little tumors (figures 11, 12, and 13). The tissue, in other words, is transplantable. In resistant chickens the implanted material survives for a few days and may be vascularized, but perishes shortly in a mass of small, round cells. The findings will not be taken up in detail since they resemble those described for Chicken Tumor I.[12]

DISCUSSION.

The growth described is in its structure, its method of enlargement, of retrogression and in its transplantability a tumor. Corroborative evidence of its neoplastic character is found in its effect on the host, in its limitation in transmissibility to one species, and in the complete natural resistance manifested by some individuals of the susceptible species. Several unsuccessful attempts have been made to cultivate from its tissue an etiological organism. The cultures either have remained sterile, or have shown diverse contaminations, such as sooner or later enter a growth transplanted without rigorous asepsis.

Every tumor has its peculiarities and in one particular Chicken Tumor XVIII is nearly unique. It tends to metastasize to the skeletal muscles, and this without localization in the viscera, and in

[11] Graf, R., *Centralbl. f. allg. Path. u. path. Anat.*, 1910, xxi, 723; Daels, F., *Arch. de méd. expér. et d'anat. path.*, 1910, xxii, 645; Levin, I., and Sittenfield, M. J., *Proc. Soc. Exper. Biol. and Med.*, 1910–11, viii, 114.

[12] Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

the absence of a patent foramen ovale or other short cut to the greater circulation. The facts might be taken to suggest that the growth's distribution occurs, not by cell emboli, but by means of some minute, infective agent, to which the pulmonary capillaries offer no barrier. As a matter of fact the paradoxical distribution of the metastases speaks neither for nor against an origin by cell emboli. It is true that pulmonary metastases are often the first step to the distribution of a growth by the greater circulation; and their presence furnishes good evidence for the dependence of this process upon cells: yet carcinoma and sarcoma both succeed rather frequently in passing the barrier of the pulmonary capillaries without the aid of lung tumors, so far as can be determined.[13] Everyone with an experience at the autopsy table has met such cases. The slight recognition accorded them in text-books perhaps results from the endeavor to present in a sharp cut way the mechanics of cell distribution and the classical picture of a tumor.

Whether Chicken Tumor XVIII really metastasizes by means of cells cannot be determined from the data at hand. The general characters of the growth place it among the tumors and these, so far as we know, can be distributed only by cells. The tendency to localize in the muscles may be likened in its strangeness to the tendency of certain human neoplasms, for example, carcinoma of the prostate, to localize secondarily in the bones. In the case of Chicken Tumor XVIII the peculiarity attracts especial attention because of the many duplicate transplantation tumors that show it.

RELATION TO CHICKEN TUMOR I.

Though Chicken Tumor XVIII in its original form had few characters in common with Chicken Tumor I,—the first growth reported upon from this laboratory,—yet with repeated transplantation it has come to resemble it somewhat. Both are now spindle-celled sarcomata, with cells of medium size and very similar appearance. Both cause the host to waste away and die in coma. Filterable agents give rise to both. Chicken Tumor I was found in a Plymouth Rock fowl and its transplantation was for a long time

[13] Zahn, F. W., *Virchows Arch. f. path. Anat.*, 1889, cxvii, 1.

most successful in fowls of this sort, while Chicken Tumor XVIII, though occurring originally in a brown Leghorn chicken, has done strikingly well in Plymouth Rocks. It may properly be asked whether Chicken Tumor XVIII does not represent a form, originally much attenuated, of the disease which we know as Chicken Tumor I.

This does not seem to be the case. Despite a heightened malignancy which has rendered it of late more nearly comparable to Chicken Tumor I, Chicken Tumor XVIII has retained distinguishing characters, as can well be seen when both tumors are growing in one host at the same rate. Chicken Tumor XVIII is then observed to be a growth of fairly homogenous structure, firm from much collagen, and rarely necrotizing or becoming hemorrhagic; Chicken Tumor I is soft, and has a structure much varied by hemorrhage, necrosis, and mucoid degeneration. The giant cells and other degenerative forms which are a striking feature of Chicken Tumor I, are not found in Chicken Tumor XVIII. In certain brown Leghorn fowls in which both metastasized, Chicken Tumor I did so to the lungs and further viscera, while Chicken Tumor XVIII localized in the muscles and there only. When Chicken Tumor I is growing slowly in partially resistant hosts it does not exhibit the characters of Chicken Tumor XVIII in the earlier slowly growing generations, but keeps its own peculiarities. Finally, artificial attenuation of the agent causing Chicken Tumor I, by heating, or agents causing the two growths may nevertheless have some relationship to each other. The point remains to be investigated. But certainly the growths to which they give rise are separate entities.

SUMMARY.

A spontaneous chicken sarcoma, peculiarly fissured by blood sinuses, and with a tendency to intracanalicular extension into them, has been transplanted and studied in eight successive groups of fowls. Histologically the growth is a characteristic neoplasm, while in its transfer to new hosts a real transplantation is obviously involved. The development of the first few series of transplantation tumors was very slow. They exhibited the histological struc-

ture of the original growth and had the same tendency to metastasize to the skeletal muscles. Recently the tumor has grown more rapidly and in a higher percentage of hosts. With this has come a simplification of structure to that of a pure, spindle-celled sarcoma.

Fowls of an alien variety (Plymouth Rock) form quite as good hosts for the tumor as those of the sort (brown Leghorn) in which it was originally found. It has not grown in pigeons, rats, or mice.

The question of the cause of the tumor is not taken up in the present paper. It has been found to be due to an agent which will pass through Berkefeld filters. The growth is quite distinct in its characters from the other two transplantable neoplasms of the fowl (a spindle-celled sarcoma, an osteochondrosarcoma) which have such a cause. No growth like it has been observed among the forty-three spontaneous tumors of the fowl that have come under our observation.

EXPLANATION OF PLATES.[14]

Plate 49.

Fig. 1. Chicken Tumor XVIII in the gizzard of the original fowl. Three metastases are visible in the skeletal muscles, namely one in the neck, another in the thoracic wall above the right lobe of the liver, and the third on the inner surface of the pelvis. All are indicated by arrows.

Plate 50.

Fig. 2. Sagittal section through the gizzard and the primary growth.

Fig. 3. Sagittal section of the left leg of the original fowl to show the tumor masses in the muscles above and below the knee. Nearly all of the non-neoplastic tissue has been cut away. The extension of the growth into the joint cavity of the knee can be plainly seen.

Plate 51.

Fig. 4. Margin of the growth in the gizzard of the original fowl. To the left are normal muscle and the mucous membrane of the gizzard cavity. The dilatation of blood spaces that precedes the invasion of the growth should be noted, and the general absence of a cellular reaction. Many of the growth's subdivisions show an interstitial hydropic change, and at the extreme right its tissue is necrotic.

Fig. 5. Section through the tumor mass in the leg of the original host, to show the fissured character of the growth. The darkly stained bodies in the vessels are the nucleated red cells of the fowl.

[14] All the microscopic sections were stained with eosin and methylene-blue.

PLATE 52.

FIG. 6. Chicken Tumor XVIII in a fowl of the second generation, dying five and one half months after inoculation. The primary masses lie in the pectoral muscle to either side of the sternum. There are large secondary tumors in the muscles of both legs and in one wing. A small tumor nodule overlies the first joint of the other wing. The emaciation of the fowl is largely concealed by the growths.

FIG. 7. Section of the tumor in a fowl of the third generation, series A. The heavy black dots scattered here and there are artefacts. The fissured, intracanalicular structure is well shown.

PLATE 53.

FIG. 8. A metastasis in the lung. The growth is here a pure, spindle-celled sarcoma. The fissured primary tumor is shown in figure 7.

FIG. 9. Chicken Tumor XVIII replacing striped muscle. The muscle fibers are split up by the growth into numerous small fragments.

PLATES 54 AND 55.

FIG. 10. Section of another part of the same tumor, showing focal, hydropic degeneration. In the undegenerated area collagen fibrils are numerous.

FIGS. 11, 12, and 13. Three grafts of Chicken Tumor XVIII shown in cross-section with the surrounding tissue. All were implanted at the same time in a susceptible, normal fowl and removed one, two, and three days later, respectively. They lie just beneath the epidermis in the pars reticularis of the corium.

The graft removed one day after implantation is unattached and its cells are living except toward the center of the fragment where necrosis is already apparent. There is some cellular reaction about the tumor bit removed at the end of the second day. Though as yet unvascularized, it is here and there attached to the host tissue, and most of its cells are living. At the end of the third day vascularization has occurred, and the tumor cells, successfully transplanted, are proliferating and extending into the host tissue.

FIG. 1.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

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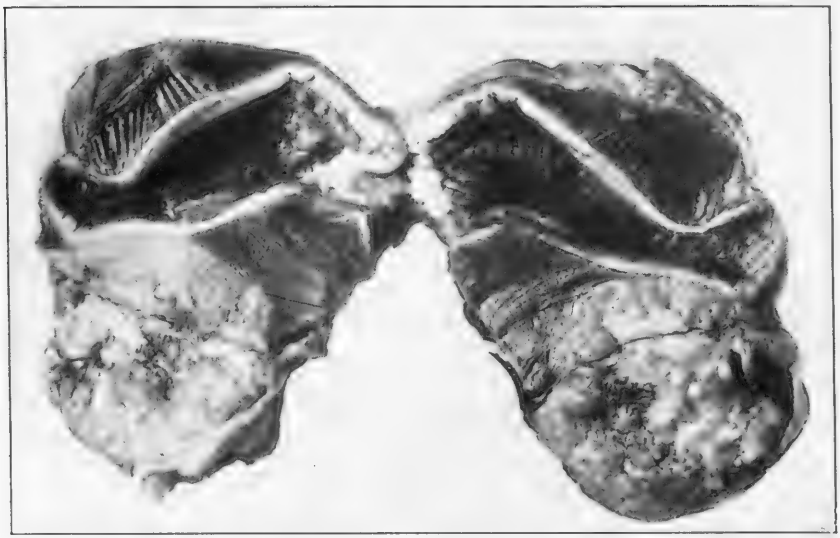
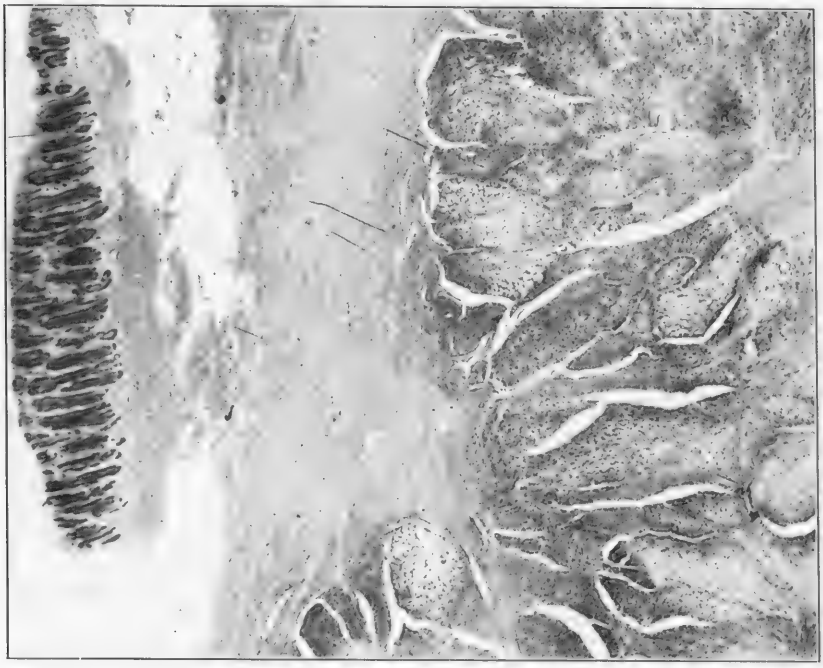


FIG. 2.

FIG. 3.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)



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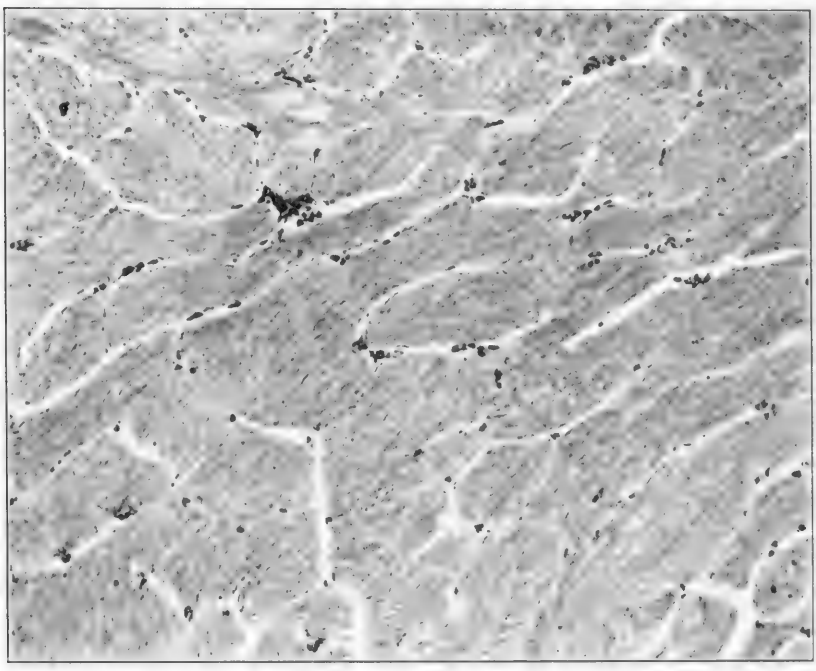

FIG. 5.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

FIG. 6.

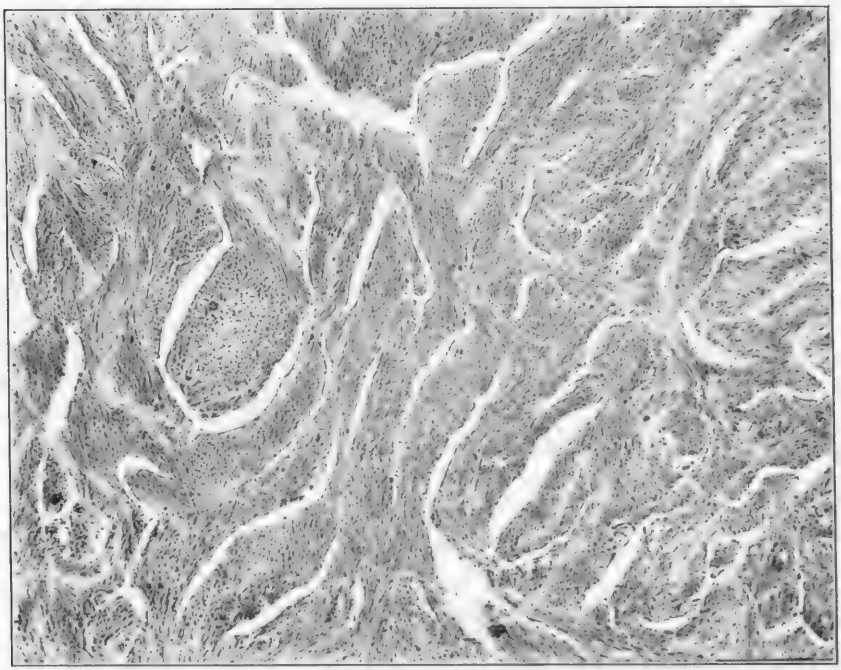

FIG. 7.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

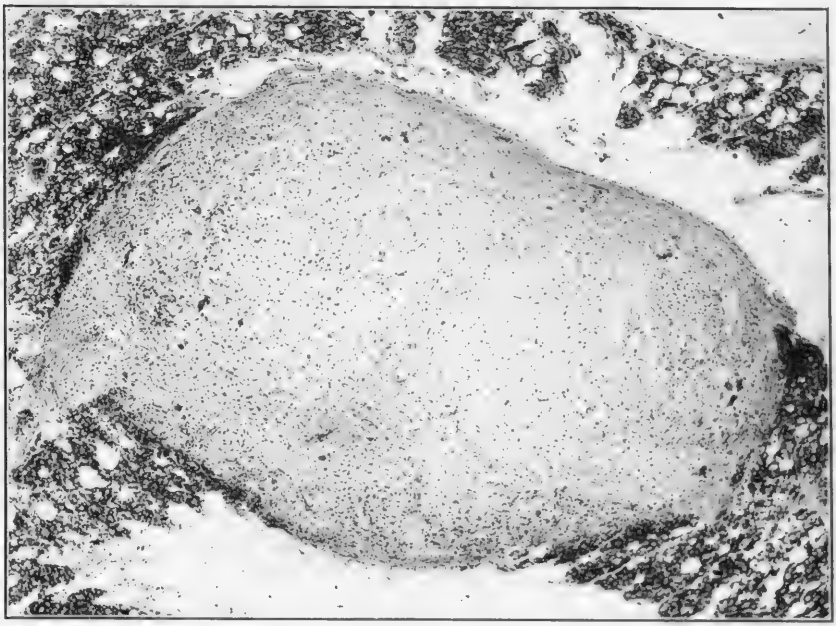

FIG. 8.

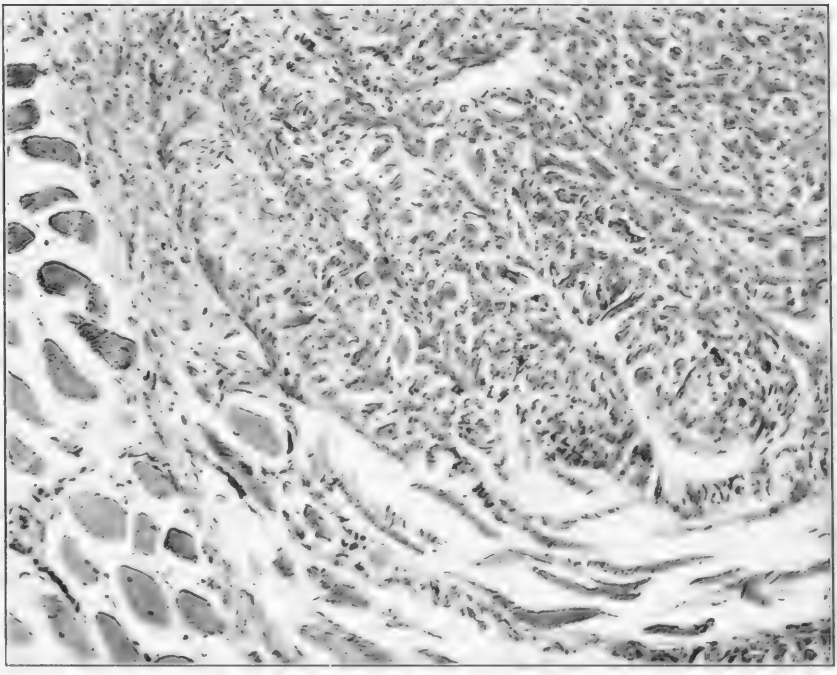

FIG. 9.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

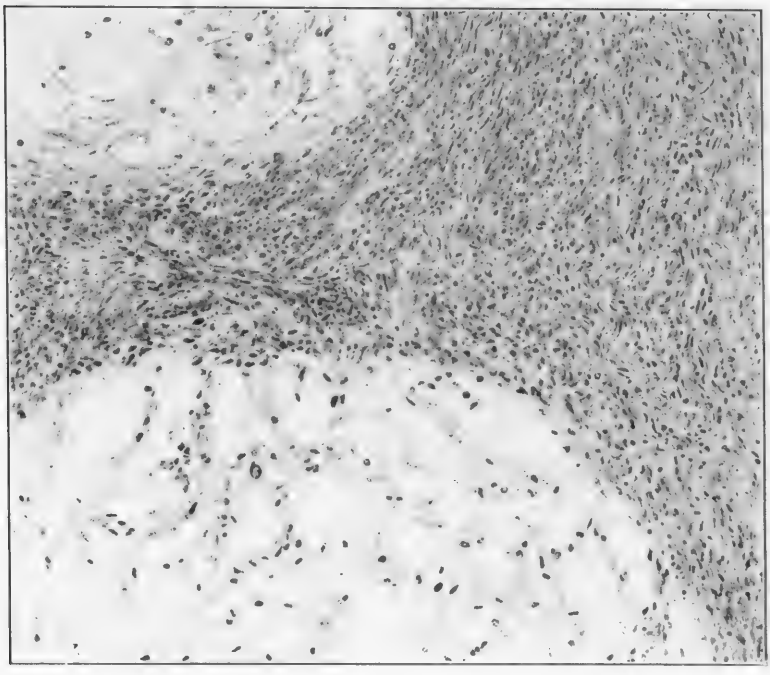

FIG. 10.

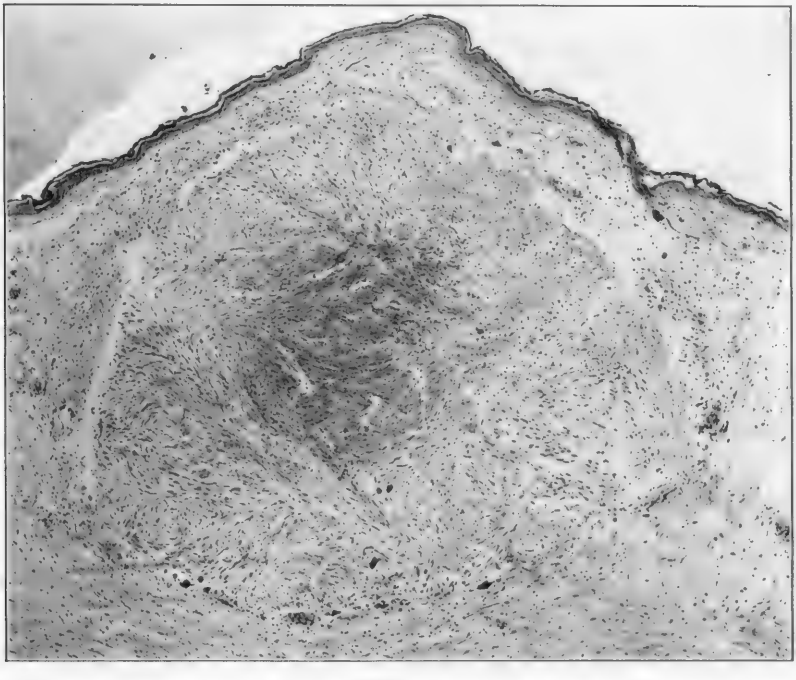

FIG. 11.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

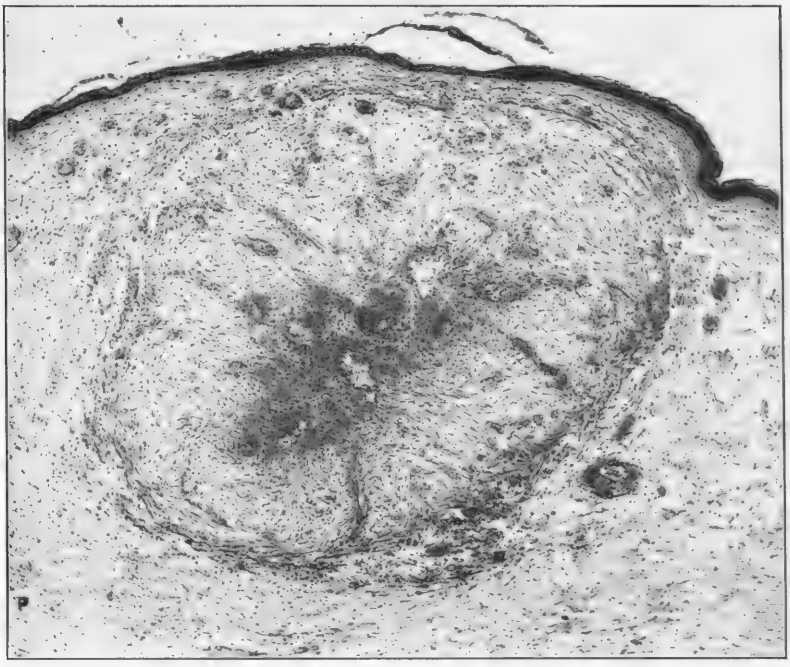

FIG. 12.

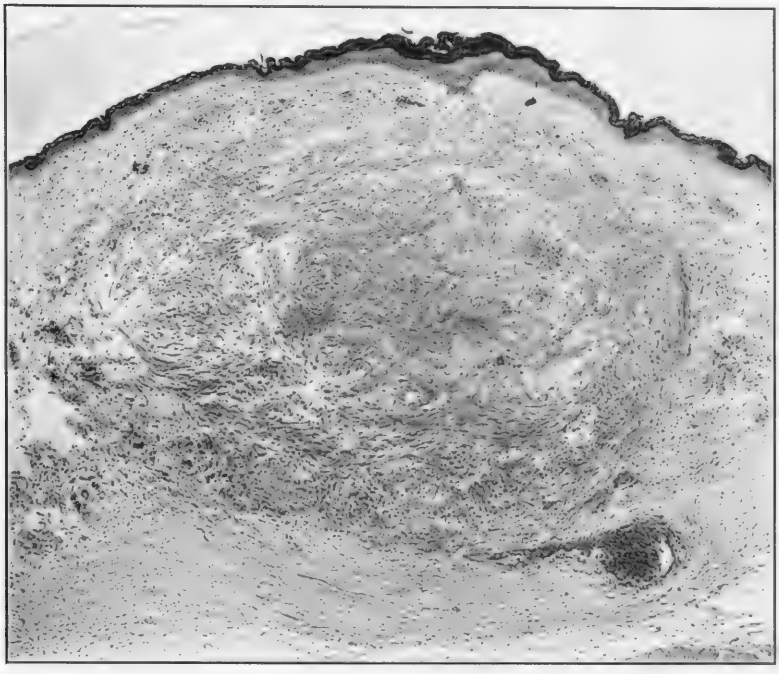

FIG. 13.

(Rous and Lange: Chicken Tumor Due to Filterable Cause.)

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

VIII. Regeneration of the Blood of Splenectomized Dogs after the Administration of Hemolytic Agents.*

By EDWARD B. KRUMBHAAR, M.D., JOHN H. MUSSER, Jr., M.D., and RICHARD M. PEARCE, M.D.

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In previous investigations on this general subject,[1] as reported from this laboratory, it has been found that in splenectomized dogs, (1) anemia, of varying grade, constantly occurs, (2) that the red cells become more resistant to hemolytic agents, and (3) that the hemoglobinemia caused by hemolytic agents is more rarely followed by jaundice than is the case in the normal dog. The cause of the anemia and the increased resistance of the red cells has not as yet been determined; it has, however, been shown that the blood of splenectomized animals returns to its normal condition after a lapse of five to ten months.[2]

This curious combination of phenomena,—that is, anemia with slow but progressive repair, increased resistance of the red corpuscles, and lessened tendency to the formation of bile pigment from free hemoglobin,—led, in the hope of eliciting new facts, to the present study of the course of the blood regeneration in animals receiving hemolytic agents at various periods after splenectomy.

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[1] *Jour. Exper. Med.*, 1912, xvi, 363, 376, 758, 769, 780; 1913, xviii, 487, 494.

[2] Musser, J. H., Jr., and Krumbhaar, E. B., VI. The Blood Picture at Various Periods after Splenectomy, *Jour. Exper. Med.*, 1913, xviii, 487.

METHODS.

The animals were given a simple, but abundant diet, practically uniform in character and containing roughly the same constant amount of iron-yielding material. The iron in the diet was not, however, in this series of experiments, estimated quantitatively.[3] After preliminary blood examinations had been made, the spleen was removed aseptically, by a practically bloodless operation, under ether anesthesia. The blood examinations were then continued, at first at shorter intervals and later after longer intervals. At various stages of the process of blood regeneration, hemolytic immune serum or sodium oleate was administered, with, at the same time, the administration of an equivalent amount of the hemolytic agent to a normal animal serving as control. For the purpose of further control animals were rendered anemic by bleeding, in order that the regeneration of the blood in the normal dog might be contrasted with that occurring in the splenectomized dog. The examination of the blood was continued after administering hemolytic agents, the intervals between examinations being gradually lengthened, and included the estimation of red cells, hemoglobin content, and the white cells with differential counts of the latter and the determination of the resistance of the red cells to various percentages of hypotonic salt solution.

RESULTS.

On account of an apparent paradox certain general results of this work must be here anticipated in order that the detailed descriptions may be better understood.

As has been shown in previous communications, not only do the red cells of splenectomized animals show an increased resistance to hypotonic salt solutions and to hemolytic immune serum, but the administration of the latter is always followed by a lesser degree of hemolytic jaundice than is the case in the normal animal. The present study, however, brings out the fact, which had been previously suspected, that despite the increased resistance of the red cells and the lesser degree or practical absence of jaundice, the ane-

[3] Experiments on the influence of the absence of the spleen on the metabolism of iron will be reported later.

mia caused in splenectomized animals by hemolytic agents is, as shown by direct blood examination, of greater severity, runs a longer course, and has a longer period of repair than is the case in the normal dog. We have therefore the apparent paradox that an animal with more resistant corpuscles suffers a severer and more prolonged anemia than an animal with corpuscles of the normal resistance. The probable interpretation of this we shall discuss later.

ANEMIA DUE TO HEMOLYTIC SERUM IN THE SPLENECTOMIZED ANIMAL.

The two experiments given in detail in tables I and II represent the longest periods that animals have been observed after the administration of hemolytic serum. In all other experiments of this group, although the animals were carried for shorter periods, the general course of the anemia was the same. The two tables are deemed sufficient, therefore, to illustrate the onset and repair in this type of anemia.

TABLE I.

Splenectomized Dog and Control. Effect of Hemolytic Serum.

Hemolytic Immune Serum 61.					
Dog 51. Weight 8,120 gm. (26 days after splenectomy.) 0.2 c.c. hemolytic serum per kilo in vein.			Dog 53. Weight 7,780 gm. 0.25 c.c. hemolytic serum per kilo injected.		
	Hemoglobin, per cent.	Red blood cells.		Hemoglobin, per cent.	Red blood cells.
Before	90	5,230,000	Before	92	6,200,000
2½ hrs.	72	3,800,000	3 hrs.	78	4,970,000
1 dy.	60	3,510,000	1 dy.	38	2,060,000
3 dys.	66	3,920,000	2 dys.	30	2,250,000
6 dys.	54	2,730,000	4 dys.	38	3,100,000
8 dys.	38	2,310,000	6 dys.	49	3,420,000
10 dys.	55	3,100,000	8 dys.	42	3,400,000
14 dys.	48	2,870,000	10 dys.	42	3,110,000
17 dys.	53	3,220,000	12 dys.	50	3,980,000
22 dys.	51	3,060,000	17 dys.	64	3,810,000
28 dys.	67	3,510,000	24 dys.	80	4,230,000
34 dys.	64	3,390,000	31 dys.	86	4,530,000
44 dys.	76	4,880,000	66 dys.	81	5,120,000
56 dys.	85	4,210,000	86 dys.	92	5,380,000
70 dys.	63	3,540,000	107 dys.	105	6,510,000
79 dys.	79	4,120,000			
100 dys.	86	4,010,000			
136 dys.	82	4,200,000			
200 dys.	110	6,200,000			

TABLE II.

Time.	Ten months splenectomy, dog 59.	Time.	Control, dog 54.
July 24, 1912	Splenectomy.		
May 21, 1913	Weight 10,900 gm. Blood: red cells 5,200,000; hemoglobin 86 per cent. Fragility: 0.3+, 0.45−. Urine: no bile pigment; trace of albumin; no casts.	May 21, 1913	Weight 12,800 gm. Blood: red cells 5,400,000; hemoglobin 104 per cent. Fragility: 0.3+, 0.5−. Urine: not obtained.
1st injection.	0.2 c.c. hemolytic serum per kilo.	1st injection.	0.2 c.c. per kilo of same serum. Urine shows faint trace of bile after 10 min.
24 hrs. later..	Blood: red cells 4,820,000; hemoglobin 90 per cent.; serum free of hemoglobin. Fragility: 0.35+, 0.6−. Urine: trace of albumin; trace of bile pigment.	24 hrs. later	Blood: red cells 5,050,000; hemoglobin 94 per cent.; serum free of hemoglobin. Fragility: 0.35+, 0.6−. Urine: trace of albumin; moderate amount of bile pigment present.
2d injection..	Same dose of same serum repeated.	2d injection..	Same dose of same serum repeated.
24 hrs. later..	Blood: red cells 5,620,000; hemoglobin 95 per cent. Urine: no hemoglobin; faint but definite trace of bile pigment; trace of albumin.	24 hrs. later..	Blood: red cells 4,880,000; hemoglobin 98 per cent. Urine: slight hemoglobinuria; large amounts of bile pigment.
2d day.....	Blood: red cells 4,030,000; hemoglobin 75 per cent. Urine: bile pigment abundant but less than in dog 54.	2d day.....	Blood: red cells 4,480,000; hemoglobin 97 per cent. Urine: no hemoglobin but large amount of bile pigment.
3d day.....	Blood: red cells 3,950,000; hemoglobin 56 per cent. Urine: good bile reaction, but less than in dog 54.	3d day.....	Blood: red cells 4,820,000; hemoglobin 95 per cent. Urine: bile very abundant.
4th day.	Blood: red cells 3,580,000; hemoglobin 52 per cent. Urine: trace of bile.	4th day.....	Blood: red cells 4,460,000; hemoglobin 66 per cent. Urine: bile decreasing, but well marked.
5th day.....	Blood: red cells 3,550,000; hemoglobin 57 per cent. Urine: bile reaction slight.	5th day.....	Blood: red cells 4,720,000; hemoglobin 74 per cent. Urine: abundant bile.
7th day.....	Blood: red cells 3,660,000; hemoglobin 52 per cent. Urine: no albumin or bile.	7th day.....	Blood: red cells 5,500,000; hemoglobin 86 per cent. Urine: moderate bile; no albumin.
11th day....	Weight: 10,040 gm. Blood: red cells 4,250,000; hemoglobin 54 per cent.; leucocytes 10,400. Urine: no albumin or bile.	11th day....	Weight 11,980 gm. Blood: red cells 5,800,000; hemoglobin 84 per cent.; leucocytes 17,000. Urine: no bile.
14th day....	Blood: red cells 4,570,000; hemoglobin 72 per cent.; leucocytes 14,200. Urine: faint trace of bile.	14th day....	Blood: red cells 5,640,000; hemoglobin 102 per cent.; leucocytes 18,200. Urine: faint trace of bile.

TABLE II.—*Continued.*

Time.	Ten months splenectomy, dog 59.	Time.	Control, dog 54.
17th day....	Blood: red cells 5,050,000; hemoglobin 86 per cent.; leucocytes 16,000.	17th day....	Blood: red cells 6,100,000; hemoglobin 106 per cent.
	Urine: no albumin or bile.		Urine: no albumin; very faint trace of bile.
20th day....	Weight 10,415 gm.	20th day....	Weight 12,365 gm.
	Blood: red cells 4,780,000; hemoglobin 79 per cent.; leucocytes 14,400.		Blood: red cells 6,080,000; hemoglobin 109 per cent.; leucocytes 20,700.
24th day....	Blood: red cells 5,440,000; hemoglobin 92 per cent.	24th day.....	Blood: red cells 6,120,000; hemoglobin 110 per cent.; leucocytes 13,200.
	Urine: no albumin or bile.		
27th day....	Blood: red cells 5,020,000; hemoglobin 90 per cent.		
31st day.....	Blood: red cells 3,480,000; hemoglobin 70 per cent.; leucocytes 13,400.	33d day.....	Blood: red cells 6,200,000; hemoglobin 110 per cent.; leucocytes 13,200.
	Fragility: 0.275+, 0.55−.		Fragility: 0.325+, 0.55−.
36th day....	Blood: red cells 4,870,000; hemoglobin 98 per cent.		
	Urine: no albumin or bile.		
40th day....	Blood: red cells 5,970,000; hemoglobin 102 per cent.		
46th day....	Weight 10,820 gm.	46th day....	Weight 12,450 gm.
	Blood: red cells 5,140,000; hemoglobin 98 per cent.		Blood: red cells 6,060,000; hemoglobin 106 per cent.
	Fragility: 0.35+, 0.475−.		Fragility: 0.35+, 0.55−.

The period intervening after splenectomy is indicated in parenthesis after the number of the animal. The word "before" in the time columns refers to the blood count made a short time before the injection of the hemolytic agent[4] and not to the blood count before splenectomy. Likewise hours and days in the same columns are indicative of the length of time after such injection.

The first experiment is of interest chiefly in connection with the question of the length of time necessary, in the splenectomized as compared with the normal dog, for a complete regeneration of the blood to occur after the destruction caused by hemolytic serum. It will be seen that in the normal dog the lowest figures were those of the fourth day, that an approach to normal figures was evident after two and three months, but that the latter were not reached until after 107 days; on the other hand, the splenectomized animal

[4] The hemolytic serum was prepared by injecting a rabbit repeatedly with 5 c.c. of dog blood at intervals of five to seven days. One week after the fifth injection the rabbit was bled and the serum collected.

reached its lowest figures on the eighth day, and still had a low hemoglobin content and red cell count at the end of 136 days. On account of the long intervals elapsing in each case before the final count, the exact dates of return to normal cannot be given, but it is clear that one and a half months after the control animal had reached its normal hemoglobin level and one month after it had gone above its normal red cell count, the blood of the splenectomized animal was far from its normal state. At the end of 200 days, however, the blood picture gave higher figures than before injection.

In this experiment the resistance of the corpuscles does not come into the question, for at the beginning of the experiment (table VI) it was the same in each animal.

As these animals were of practically the same weight, and as the splenectomized dog received less serum than the normal one, it is impossible to escape the conclusion that the absence of the spleen is an important factor in the chronicity of the anemia and the slow repair.

In the second experiment, the same difference in the severity of the anemia and the rate of recovery is seen, but in neither animal was the period of recovery as long as in the animals represented in table I.

Some objection might be raised against the results in the first experiment, as this animal, despite the high initial count, had been splenectomized only twenty-six days, and the resulting slow repair might be due to a combination of a slow anemia secondary to absence of the spleen and of the anemia due to the hemolytic serum. This possible objection is removed by the results in the second experiment in which the blood of an animal splenectomized nearly a year before had returned to normal before injection. The theory that the absence of the spleen is responsible for the slow blood regeneration is thus strengthened.

SODIUM OLEATE ANEMIA IN THE SPLENECTOMIZED ANIMAL.

On account of the advisability of determining the effect of hemolytic poisons other than hemolytic immune serum, two experiments with sodium oleate were carried out (table III).

TABLE III.

Repair of Sodium Oleate Anemia after Splenectomy, with Control.

Dog 24. Weight 9,650 gm. (5 months after splenectomy.)				Dog 55. Weight 7,860 gm. Normal.			
100 c.c. of 1 per cent. sodium oleate in vein.				100 c.c. of 1 per cent. sodium oleate in vein.			
	Hemoglobin, per cent.	Red blood cells.	Leucocytes.		Hemoglobin, per cent.	Red blood cells.	Leucocytes.
Before	89	5,650,000	10,600	Before	108	6,460,000	8,600
1 dy.[5]	68	3,770,000	15,600	3 dys.[6]	93	5,420,000	7,100
3 dys.	70	2,320,000	10,400	7 dys.	112	6,120,000	6,400
7 dys.	74	3,100,000	10,800	16 dys.	116	7,240,000	10,800
11 dys.	70	4,200,000	8,600	24 dys.	114	7,410,000	8,200
16 dys.	66	3,420,000	9,800				
19 dys.	66	3,760,000	10,200				
24 dys.	63	3,600,000	10,000				
30 dys.	54	3,700,000	7,600				
35 dys.	63	3,890,000	10,000				
39 dys.	66	4,180,000	9,300				
44 dys.	76	4,220,000	12,800				
51 dys.	79	4,400,000	13,600				
57 dys.	86	4,820,000	8,900				
73 dys.	90	5,780,000	12,600				
200 dys.	110	6,048,000	———				

Here also it is seen that the more severe anemia and the slower repair occurs in the splenectomized rather than in the normal dog. In the first experiment the splenectomized dog's hemoglobin fell eventually, on the thirtieth day, to 54 per cent., and the red cells to 3,700,000, and it took forty-three days for the return to normal, whereas in the control receiving an equivalent amount of oleate, the hemoglobin fell on the third day to only 93 per cent., and the erythrocytes to only 5,420,000, and the return to normal, occurred in four days. In a second experiment the same general results were obtained but in less striking form. In this type of anemia it is therefore also evident that absence of the spleen delays the regeneration of the blood.

REPAIR OF BLOOD IN THE SPLENECTOMIZED DOG AFTER HEMORRHAGE.

A third method of study was to produce anemia by hemorrhage and to observe the rapidity of repair in the splenectomized as contrasted with the normal animal (table IV).

[5] Hemoglobin in urine on 1st and 3d days.

[6] Hemoglobin in urine on 3d day.

TABLE IV.

Repair after Hemorrhagic Anemia in a Splenectomized Animal, with Control.

Dog 42. Weight 8,020 gm. (85 days after splenectomy.)				Dog 55. Weight 7,860 gm. Normal.		
100 c.c. of blood taken from a vein.				150 c.c. of blood taken from a vein.		
	Hemoglobin, per cent.	Red blood cells.	Leucocytes.	Hemoglobin, per cent.	Red blood cells.	Leucocytes.
Before	92	5,160,000	22,400	95	5,200,000	10,200
1 dy.	78	4,570,000	26,200	78	5,100,000	8,300
4 dys.	68	4,310,000	24,100	78	4,900,000	10,500
8 dys.	76	3,820,000	22,100	105	6,120,000	10,400
12 dys.	85	4,380,000	16,800	——	——	——
18 dys.	84	4,210,000	16,100	——	——	——
26 dys.	70	4,850,000	17,400	108	6,460,000	8,600
34 dys.	74	4,100,000	12,600			
40 dys.	90	4,480,000	10,200			
46 dys.	93	4,390,000	16,400			
54 dys.	95	4,760,000	13,100			
61 dys.	90	4,850,000	14,900			
67 dys.	102	5,100,000	19,200			

Here it is seen that a small loss of blood causes a graver anemia in the splenectomized than in the normal dog and that repair is greatly delayed. After a loss of 100 cubic centimeters of blood the hemoglobin of the splenectomized dog was lowered on the fourth day to 68 per cent., and the erythrocytes to 4,310,000, with a return to normal sixty-three days later. From the control dog of slightly less weight, 150 cubic centimeters of blood were taken, the hemoglobin dropping to 78 per cent. and red cells to 4,900,000 on the fourth day and promptly returning to normal within four days.

THE LEUCOCYTES.

The behavior of the leucocytes in many of the experiments has not been constant. This is probably due to the fact that in experiments over such long periods it is not always possible to avoid mild intercurrent bacterial infections or infections by intestinal parasites, or to recognize them when they occur. Our somewhat discordant results with the leucocyte counts allow therefore no definite conclusion in regard to the animals receiving sodium oleate and those in which anemia was produced by hemorrhage, largely because only a few animals were used in these two groups. On the other

hand, in the group receiving hemolytic serum, on account of the large number of examinations made, it may be said definitely that hemolytic serum causes a constant leucocytosis, due to an increase in the polymorphonuclear neutrophils, and that this usually reaches a higher grade in the splenectomized dog than in the normal dog. Both show a moderate increase in lymphocytes, which persists for long periods. A persisting increase of eosinophils occurs in the splenectomized dog.

In this connection we insert one table (table V) showing the course of the leucocytosis and the results of differential counts on two of the animals (51 and 53) previously presented in table I.

TABLE V.

Leucocyte and Differential Counts after Hemolytic Serum.

Dog 51. (26 days after splenectomy.)					Dog 53. Normal.				
	Leucocytes.	Polymorphonuclear cells, per cent.	Small lymphocytes, per cent.	Eosinophils, per cent.		Leucocytes.	Polymorphonuclear cells, per cent.	Small lymphocytes, per cent.	Eosinophils, per cent.
Before	24,200	86	9	3	Before	24,000	71	12	6
1 dy.	18,600				1 dy.	24,000	92	4	1
2 dys.	56,000				4 dys.	28,600			
3 dys.	28,400	73	8	12	8 dys.	36,000			
6 dys.	52,000				10 dys.	38,400	81	11	0
8 dys.	51,000	85	6	5	12 dys.	31,200	76	18	2
11 dys.	42,400	73	13	8	14 dys.	18,200	68	21	2
14 dys.	31,100	63	25	5	17 dys.	17,800	73	24	1
17 dys.	17,200				20 dys.	13,200	60	32	2
28 dys.	13,000	54	20	20	24 dys.	14,100	66	26	5
41 dys.	18,000	60	20	16	28 dys.	19,100			
51 dys.	16,800	54	20	17	31 dys.	11,900	61	28	5
62 dys.	18,000	65	17	14	44 dys.	16,100			
79 dys.	17,200	51	24	20	59 dys.	11,200			
93 dys.	16,400				66 dys.	25,000	78	15	5
100 dys.	12,200				72 dys.	22,400			
136 dys.	10,000	50	23	24	86 dys.	19,100	67	22	2
					95 dys.	12,800			
					107 dys.	10,200			
Normoblasts and polychromatophilia constantly present until 28th day. One normoblast seen in last count.					Normoblasts present for 17 days, none thereafter. Polychromatophilia less marked than in dog 51.				

RESISTANCE OF THE RED CELLS TO HYPOTONIC SALT SOLUTION AFTER THE ADMINISTRATION OF A HEMOLYTIC AGENT.

In an earlier communication[7] it was shown that the increased resistance of the red blood cells of a splenectomized animal to the action of hypotonic salt solution and to hemolytic serum is "not due to an increased antihemolytic power of the animal's serum or to a diminished complementary value of the serum, but is a property dependent upon the erythrocytes themselves." In the course of the present investigation it was noted that shortly after the administration of hemolytic serum the cells of the splenectomized animals, instead of being more resistant to hypotonic solution, became less resistant and that this decreased resistance persisted in the splenectomized animal for a longer period than in the normal animal. This is shown in the following abridged table.

TABLE VI.[8]

Resistance of Red Blood Corpuscles after Injection of Hemolytic Serum.

Dog 51. (26 days after splenectomy.)	Per cent. salt solution.							
	0.25	0.3	0.35	0.4	0.45	0.5	0.55	0.6
Before serum was administered	+	+	P	P	O	O	O	O
3 hrs. after serum was administered	+	+	P	P	P	O	O	O
6 dys. after serum was administered	+	+	P	P	P	P	P	P
8 to 34 dys. after serum was administered	+	+	P	P	P	P	P	P
41 to 76 dys. after serum was administered	+	+	P	P	P	O	O	O
100 dys. after serum was administered	+	+	P	P	O	O	O	O
Dog 53. Normal.								
Before serum was administered	+	+	P	P	O	O	O	O
18 hrs. after serum was administered	+	+	P	P	P	P	P	O
8 to 12 dys. after serum was administered	+	+	P	P	P	P	P	P
20 to 95 dys. after serum was administered	+	+	P	P	P	O	O	O

Here it is seen that the erythrocytes of the splenectomized and the normal dog had the same resistance to salt solution before the injection, and likewise the same decrease in resistance eight days after the injection of hemolytic serum. The cells of the normal animal, however, returned to almost normal resistance after twenty

[7] Karsner, H. T., and Pearce, R. M., IV. A Study, by the Methods of Immunology, of the Increased Resistance of the Red Blood Corpuscles after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 769.

[8] + indicates complete hemolysis; P, partial hemolysis; and O, no hemolysis.

days, while those of the splenectomized animal did not return to the same degree until the forty-first day. On the other hand, it will be seen that the splenectomized animal returned to the original point after one hundred days while the normal animal failed to do so after ninety-five days.

In the anemia produced by sodium oleate and by hemorrhage the decreased resistance, although it occurs, is not so striking in either the normal or the splenectomized animal as it is in the animals receiving hemolytic serum; the splenectomized animal, however, always shows a greater decrease in resistance than does the normal.

The observation is of interest in connection with the theories of Port[9] who considers that certain hemolytic poisons as sapotoxin act directly on the bone marrow so injuring it that corpuscles of lessened resistance, and therefore capable of hemolysis in higher strengths of salt solution, rapidly enter the blood stream while poisons of another type, as benzidin, attack directly the erythrocytes in the circulating blood and thus stimulate indirectly the bone marrow to the production of more resistant cells. Hemolytic serum, as has been shown in the course of this general investigation,[10] causes eventually an increased resistance of the cells of the normal dog and probably belongs to Port's second group. Nevertheless there is no doubt that for a considerable period after injection the cells are less resistant than normally. The exact relation of the various types of hemolytic poisons to degree of blood degeneration and regeneration after splenectomy is a question we now have under investigation. Here it may suffice to say that the absence of the spleen appears to be an important factor in causing the persistence of the lessened resistance of the red cells after the use of hemolytic serum, and therefore is a factor in the production of the more severe and more prolonged anemia which this serum causes in splenectomized, as contrasted with normal animals.

SUMMARY.

In the splenectomized dog the anemia caused by hemolytic poisons (hemolytic immune serum and sodium oleate) and by bleeding is

[9] Port, F., Die Resistenz der Erythrozyten, *Arch. f. exper. Path. u. Pharmakol.*, 1912, lxix, 307.

[10] Karsner, H. T., and Pearce, R. M., *loc. cit.*

of a severer grade, runs a longer course, and is accompanied by a less rapid regeneration of the blood than is the case in the normal dog. Also in the splenectomized dog, especially after the use of hemolytic serum, the leucocytosis is greater than in the normal animal.

The splenectomized dog almost uniformly exhibits an increased resistance of the red cells to hypotonic salt solution, but after the administration of hemolytic poisons, and especially hemolytic serum, this increased resistance disappears and a decreased resistance persists for long periods of time. The same change occurs in the normal dog, but in the latter the return to the previous degree of resistance is more rapid than in the splenectomized animal.

DISCUSSION.

At first glance these results appear to contradict some of our earlier work, in which it was shown that the red cells of the splenectomized animal are more resistant to hemolytic agents than is the normal dog, and that in such, hemolytic jaundice is not so readily produced. On the basis of these observations, concerning the accuracy of which repeated experiments have furnished a large mass of corroborative evidence, one would hardly expect a hemolytic poison to cause in the splenectomized animal a more severe anemia than occurs under similar circumstances in the normal animal. The present investigation, however, shows this to be the case, and, moreover, that hemolytic poisons change the more resistant cells of the splenectomized to less resistant cells, this diminished resistance persisting longer than in the normal dog.

It is evident therefore that the immediate destruction of the red cells by a hemolytic agent, as shown by hemoglobinuria and the result of the blood examination, is not the only factor in the production of the anemia, but that in the splenectomized animal some added factor plays an important part. The hemolytic agent inaugurates an acute anemia, but the absence of the spleen is an important secondary factor in increasing the severity of the anemia and in either prolonging its course or delaying the process of repair.

It would appear therefore that of the phenomena associated with

the absence of the spleen, two, the increased resistance of the red cells and the decreased tendency to jaundice after the administration of hemolytic poisons, are co-related and intimately associated with the anemia of splenectomy, but that the anemia itself is dependent upon some factor as yet unknown, which operates in the absence of the spleen. This unknown factor would also operate in the anemia produced in such animals by hemolytic serum, and more especially so, as the hemolytic anemia is characterized by a decreased resistance of the red cells. As the factor dependent on the absence of the spleen does not operate in the normal animal, the latter recovers quickly. It seems possible therefore thus to explain the more severe and more prolonged anemia in such animals, even though they may have more resistant corpuscles.

THE PRODUCTION OF EXPERIMENTAL NEPHRITIS BY REPEATED PROTEID INTOXICATION.*

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Plates 56 to 65.

During the last two years there have been under observation at the Presbyterian Hospital occasional cases of nephritis, characterized by exacerbations of general edema, fever, albuminuria, and, in a few instances, urticaria and eosinophilia, which in these particular attacks, have a close resemblance to serum disease. It was through the suggestion made by other workers, and derived from a study of these cases, namely, that nephritis might in certain instances be dependent for its origin upon an anaphylactic state, that the present investigation was undertaken.

Comparatively little attention has been paid to the anatomical alterations that may take place during anaphylactic shock, and almost no observations have been made upon the effects of repeated non-fatal anaphylactic shock upon the animal tissues. It is known that man may develop a hypersensitiveness, or allergy, as von Pirquet calls it, to some food stuffs, among which may be mentioned egg-white, fruits, and shell-fish, and under these circumstances it is possible that he may be subjected from time to time to many non-fatal attacks of intoxication by the proteins of these substances. It is important, therefore, to determine if in animals artificially sensitized to foreign protein, repeated intoxications of these foreign proteins may cause injurious effects upon the body.

Recently the question that has absorbed the interest of most investigators is the manner in which the hypothetical intoxicating substance, anaphylatoxin, is

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formed during the anaphylactic shock. The views formulated by Vaughan,[1] Biedl and Kraus,[2] Friedberger,[3] and Schittenhelm and Weichard,[4] and well summarized by Friedemann,[5] tend to explain the phenomenon by the formation, during the period of sensitization, of a ferment, or of an antibody towards the foreign substance, which, at the time of the second injection of protein, two or three weeks after sensitization, acts upon this protein in such a manner that it is split into substances that are simpler chemically, but, like peptones, highly toxic. Vaughan considers that this process takes place within the body cells by the action of an intracellular ferment, but the more common conception is that of antibody formation such as takes place in the production of agglutinins or precipitins.

Attempts have been made to discover whether these antibodies reside in the cells of various organs; and discussion has arisen as to whether the liberation of the toxic substance takes place in the cells of organs or in the circulation. So far, with one or two questionable exceptions, investigators have failed to demonstrate antibodies for foreign protein in any of the cells of the sensitized animals. Recently, however, Felländer and Kling[6] have shown that antibodies may be found in the bone marrow and leucocytes of sensitized animals. On the other hand, it has been shown repeatedly by various methods that the serum of sensitized animals contains antibodies that will act upon the specific protein to which the animal is sensitive to form highly toxic substances (Friedberger). There are, however, certain observations which go to show that a reaction may take place during anaphylaxis between the foreign protein and the body cells; for the experiments of Schultz,[7] and particularly their amplification by Dale,[8] demonstrate definitely that the smooth muscle of the uterus, intestine, and bronchi, washed free of blood, from the guinea pig sensitized to horse serum will contract as it does *in vivo* during anaphylaxis, when brought into contact *in vitro* with dilutions of horse serum which have no effect upon the musculature of the organs from normal guinea pigs.

The actual anatomical changes in guinea pigs dying acutely in anaphylaxis have been studied extensively by Gay and Southard.[9] They describe hemor-

[1] Vaughan, V. C., Cumming, J. G., and McGlumphy, C. B., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 16. McFarland, W. L., *ibid.*, 458. Vaughan, V. C., Vaughan, V. C., Jr., and Wright, J. H., *ibid.*, xi, 673.

[2] Biedl, A., and Kraus, R., *Wien. klin. Wchnschr.*, 1909, xxii, 363.

[3] Friedberger, E., and Goldschmidt, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 369. Friedberger, E., and Hartoch, O., *idem*, 1909, iii, 581. Friedberger, E., and Gröber, A., 1911, ix, 216. Friedberger, E., and Nathan, E., *ibid.*, 567.

[4] Schittenhelm, A., and Weichardt, W., *Ztschr. f. exper. Path. u. Therap.*, 1912, x, 412, 448; xi, 69.

[5] Friedemann, U., *Jahresber. ü. d. Ergebn. d. Immunitätsforsch.*, 1910, vi, 31.

[6] Felländer, J., and Kling, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1912, xv, 409.

[7] Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1909–10, i, 549; 1910–11, ii, 221.

[8] Dale, H. H., *Jour. Pharmacol. and Exper. Therap.*, 1913, iv, 167.

[9] Gay, F. P., and Southard, E. E., *Jour. Med. Research*, 1907, xvi, 143; 1908, xviii, 407.

rhages in the heart muscle, pleura, stomach wall, and cecum, and have called attention particularly to a fatty degeneration of the capillary endothelium which they believe is the direct cause of the hemorrhages. Fatty degeneration was also observed in the nerves, epithelium of the stomach, heart, and voluntary muscles. Anderson and Rosenau[10] in repeating this work lay but little stress upon the hemorrhages and believe that the occasional fatty changes they found may occur in other conditions and are not characteristic of the anaphylactic intoxication. These changes described by Gay and Southard may or may not be characteristic of anaphylaxis in the guinea pig, but they occur in my experience quite frequently, though of course the acute emphysema to which Auer and Lewis[11] first called attention is constant. By repeating the shock two or three times in guinea pigs by intraperitoneal injections Gay and Southard[12] failed to produce chronic changes.

Under certain circumstances, nevertheless, the body cells may react with resulting anatomical changes towards repeated doses of foreign protein. Thus the local edema, inflammation, and necrosis that is seen in the skin of a rabbit after subcutaneous inoculations of horse serum, repeated at intervals of six to seven days, and studied by Arthus,[13] is familiar. Lucas and Gay[14] have noted the same thing in children receiving repeated injections of diphtheria antitoxin. Another evidence of local reaction is the cellular pneumonia described by Friedberger,[15] which he was able to produce in the lungs of sensitized guinea pigs by spraying horse serum into the trachea. Ishioka[16] has repeated and confirmed these experiments, and Schlecht and Schwenker[17] have made a careful histological study of the lungs in this condition. They describe the proliferation of cells lining the alveolar walls, and the exudation of eosinophilic leucocytes into the alveoli with the formation of fibrin which gives rise to a type of bronchopneumonia. Moreover, they have studied the effects of repeated intraperitoneal inoculations of horse serum in guinea pigs as well as the Arthus phenomenon and find under these circumstances infiltrations of the omentum and peritoneal cavity in the one instance and infiltration of the subcutaneous tissues in the other by eosinophilic and small mononuclear cells. Intravenous injections of serum in dogs resulted in an intestinal catarrh with infiltration of the mucosa and submucosa of the intestines by many cells among which were eosinophils.

There is, therefore, a certain amount of evidence to show that during anaphylaxis some of the body cells may be injured, and a

[10] Rosenau, M. J., and Anderson, J. F., *Jour. Med. Research,* 1908, xix, 37.

[11] Auer, J., and Lewis, P. A., *Jour. Exper. Med.,* 1910, xii, 151.

[12] Gay, F. P., and Southard, E. E., *Jour. Med. Research,* 1908, xix, 17.

[13] Arthus, M., *Compt. rend. Soc. de biol.,* 1903, lv, 817. Arthus, M., and Breton, M., *ibid.,* 1478. Arthus, M., *Arch. internat. de physiol.,* 1908–09, vii, 471.

[14] Lucas, W. P., and Gay, F. P., *Jour. Med. Research,* 1909, xx, 251.

[15] Friedberger, E., *Deutsch. med. Wchnschr.,* 1911, xxxvii, 481.

[16] Ishioka, S., *Deutsch. Arch. f. klin. Med.,* 1912, cvii, 500.

[17] Schlecht, H., and Schwenker, M. G., *Deutsch. Arch. f. klin. Med.,* 1912, cviii, 405.

few observations tend to support the idea that this injury may be followed by a reaction on the part of the fixed and migratory body cells. A series of experiments, therefore, was planned to determine if, during repeated anaphylactic shocks in animals, any permanent injury might be done to the body cells, particularly the kidney, and whether there might be any reaction visible microscopically on the part of the tissues to such repeated intoxications.

METHODS.

For the experimental investigation of the problem several species of animals were used as one species might react differently from another; therefore guinea pigs, rabbits, cats, and dogs were selected. Horse serum and egg-white were chosen as foreign proteins. Guinea pigs were sensitized by the intraperitoneal injection of 0.01 to 0.1 of a cubic centimeter of horse serum and 0.5 of a cubic centimeter of undiluted egg-white. In order to render rabbits sensitive much larger and repeated doses were required and consequently two to three doses of the protein were injected at two to three day intervals. As a rule, one to three cubic centimeters of the protein were given intravenously, intraperitoneally, subcutaneously, or by a combination of these routes. The cats were injected once with one to four cubic centimeters of horse serum or egg-white either intravenously or subcutaneously. The dogs, according to their weight, were usually given three to five cubic centimeters of the protein either subcutaneously or intravenously. In some experiments the animals were sensitized both to horse serum and egg-white in order that a single animal might receive the greatest possible number of intoxicating doses.

Twenty to twenty-five days after the preliminary inoculation, the secondary series of injections was started and thereafter injections were made at two to three day intervals in some experiments, at five to seven day intervals in others, and in still a third group at two to three week intervals. In the guinea pigs the intoxicating doses were given intraperitoneally, and the same method as well as an occasional subcutaneous inoculation was used with a few rabbits and cats, but by far the greatest number of rabbits, cats, and

dogs received intravenous inoculations. In cats the injections were made into the ear vein, in dogs into the external jugular or saphenous veins.

It was at first difficult to determine the dose of horse serum or egg-white which would cause definite symptoms without fatal shock and consequently several animals were sacrificed before the proper dosage could be arrived at. Another difficulty that was encountered was the development of an anti-anaphylactic state. This was most rapidly acquired in guinea pigs. They ceased to show symptoms usually after the second or third intraperitoneal inoculation. Rabbits and cats, however, could be given doses of horse serum increasing from 0.1 to 2 to 4 cubic centimeters on six or eight separate occasions before they ceased to react, and in the same manner dogs received doses of horse serum up to ten cubic centimeters before they became insensitive. To large amounts of egg-white the rabbits and cats were never refractory. Dogs and guinea pigs reacted to egg-white as they did to horse serum.

The symptoms following the intoxicating doses in guinea pigs consisted of scratching at the nose, ruffling of the hair, defecation and urination, collapse with partial or complete paralysis of the hind legs, with cramp-like convulsions and complete paralysis in the severest cases. The rabbits showed preliminary excitement, followed by weakness and collapse. The animals flattened their bellies against the floor, lay with their heads back, passed urine and feces, and showed rapid respirations. In the severest shock the rabbits lay upon their sides, occasionally showed spasmodic running movements, or during their muscular contractions flopped about the table. The majority of the animals that reached this stage died. In the acutely fatal cases the animals would run across the floor, then fall on their sides, show the running motions, perhaps scream, and with bulging eyes and dilated pupils die in extreme spasm with marked opisthotonos. The cats regularly became excited, ran a short distance, then swayed for a moment and fell upon their sides. Immediately there were violent muscular contractions and the animals stiffened out in extreme opisthotonos. After half a minute to a minute they would relax and lie sprawled out in collapse. Urina-

tion, repeated defecation with bloody movements, and occasionally vomiting would follow. During this time there were contractions of the abdominal muscles and rapid respirations. After a period of ten to twenty minutes the cats would recover, and crawl to a corner where they sat quietly huddled up for some time.

The dogs after a preliminary short excitement vomited, urinated, swayed, stood with their heads hung down, and then lay upon their sides, completely relaxed and collapsed, but evidently quite conscious. A diarrhea often severe with blood and mucus followed. This condition lasted for ten to thirty minutes depending upon the severity of the attack. There were spasmodic contractions of the abdomen. Three dogs developed on several occasions severe generalized urticaria with extreme itching. Though the temperature of these animals was not regularly observed, it was recorded in a few instances and in all cases when definite shock was produced the temperature fell 1° to 1.5° C.

It may therefore be seen that these symptoms accord well with the clinical picture of anaphylactic shock as described for guinea pigs by Rosenau and Anderson,[18] for rabbits by Arthus,[19] Friedberger,[20] and Auer,[21] for cats by Schultz,[22] and for dogs by Biedl and Kraus,[23] and Pearce and Eisenbrey.[24]

The experiments have shown that repeated injections of egg-white and horse serum bring about serious damage to the cells of various organs and tissues in all these animals. A single intraperitoneal inoculation in guinea pigs, rabbits, and cats produced no local reaction on the part of the omentum or peritoneum, but after the repeated intraperitoneal injections in sensitized animals, the omentum and peritoneal tissues at autopsy appeared rather thick, opaque, and white, and microscopical examination showed that this appearance was due to the presence of chronic inflammatory proc-

[18] Rosenau, M. J., and Anderson, J. F., *Hygienic Laboratory Bulletins*, 1906, No. 29; 1907, No. 36; 1908, No. 45.

[19] Arthus, M., *Arch. internat. de physiol.*, 1908–09, vii, 471; 1910, ix, 156.

[20] Friedberger, E., and Gröber, A., *Ztschr. f. Immunitätsforsch., Orig.*, 1911, ix, 216.

[21] Auer, J., *Jour. Exper. Med.*, 1911, xiv, 476.

[22] Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1912, iii, 299.

[23] Biedl, A., and Kraus, R., *Wien. klin. Wchnschr.*, 1909, xxii, 363.

[24] Pearce, R. M., and Eisenbrey, A. B., *Jour. Infect. Dis.*, 1910, vii, 565.

esses in these tissues. The fat of the omentum in guinea pigs was infiltrated with great numbers of eosinophilic cells, together with small round cells and fibroblasts. In the rabbits and cats eosinophilic cells were rare, but the peritoneal tissue showed a chronic inflammation consisting of the presence of very cellular connective tissue which thickened the peritoneal surfaces and often converted the folds of omentum into cellular fibrous knob-like processes (figure 1).

In the lungs of guinea pigs which had received only repeated intraperitoneal injections, changes similar to those described by Friedberger, Ishioka, and Schlecht and Schwenker were found, though they were less extensive and presented the appearance of small bronchopneumonic patches and peribronchial infiltrations rich in eosinophils.

In the livers of rabbits and cats there were seen areas of necrosis often periportal in situation which could be traced in many animals to undergo organization by growth of connective tissue proceeding from the portal spaces. This led to a mild grade of portal cirrhosis which has been described in detail elsewhere[25] (figure 2).

Similar necroses were likewise observed in the myocardium in each group of animals, but most frequently in rabbits (figure 3). These necroses, too, healed with the formation of scars which in the animals that had received the greatest number of injections led to a focalized and occasionally diffuse chronic myocarditis.

In a large proportion of all animals changes were also found in the kidneys. They occurred in 79.3 per cent. of the twenty-nine inoculated rabbits, in seven of the twelve cats, in all of the thirteen guinea pigs, and in ten of the twelve dogs. Since the changes were practically the same in all groups of animals, the following description will apply to all the experiments.

When the animals died acutely the cells of the convoluted tubules showed extensive cloudy swelling and there was extreme congestion, especially marked in the glomeruli. In some of the rabbits and guinea pigs the tissues about the papilla were edematous.

Besides the diffuse degenerative changes, other microscopic lesions which appeared to be the earliest evidence of any destructive change

[25] Longcope, W. T., *Tr. Assn. Am. Phys.*, 1913, xxviii (in press).

in the kidney were seen in many rabbits and occasionally in cats, guinea pigs, and dogs that had received several intoxicating injections. These consisted of small areas in the papilla, the midzone, and the cortex in which the cells lining tubules showed necrosis and desquamation. In the papilla the cells lining the collecting tubules were affected (figure 4), in the midzone the epithelium of the loops of Henle, and in the cortex of the convoluted tubules the epithelium was involved (figure 5). The nuclei of these cells appeared pyknotic or fragmented, the protoplasm swollen and frayed, and the entire cell was loosened from the basement membrane and had a distorted appearance. Occasionally the lumen of the tubule was filled with desquamated cells. In the smallest areas the surrounding capillaries were filled with small mononuclear cells. In larger areas a single collecting tubule or groups of convoluted tubules formed the center of a dense area of round cell infiltration where the round cells were collected in great numbers between these degenerating tubules (figure 7). At the same time collections of round cells were observed about the vessels in the intermediate zone and sometimes surrounding the vessels as they ran into the cortex. These foci varied considerably in number. Occasionally there were ten to fifteen in a section, and in other instances only one or two were found.

The majority of the animals, especially the rabbits, showed what was taken to be a more advanced stage of the same process. The kidneys in some instances appeared normal on macroscopic examination. In other instances minute pale areas could be made out in the cortex or pale striæ radiated from the midzone towards the capsule. In three rabbits the kidney appeared swollen and pale, but the surface was smooth. On section there was marked swelling of the papilla. The boundary between the papilla and cortex was ill defined, while the cortex looked pale and the normal markings were practically obscured. In the dogs grayish streaks were sometimes observed in the cortex and the arcuate vessels, or the tissues about them appeared abnormally dense and pale.

On microscopical examination the kidneys presented extensive changes. Masses of round cells in enormous numbers infiltrated the intertubular tissue extending in rays from the intermediate zone

towards the capsule, occurring in great patches in the papilla or running from the intermediate zone down into the papilla. More or less focalized areas of round cell infiltration could also be seen in the cortex. In the largest areas the arrangement of the tubules could scarcely be made out and frequently it was only possible to see here and there a few epithelial cells, sometimes shrunken into small clumps which showed the remnants of tubules (figures 6 and 8). Occasionally groups of tubules in the cortex showed marked dilatation and in the lumen were hyaline casts. In these areas it could be seen that the round cell infiltration was accompanied by an increase in connective tissue which appeared as a rather delicate meshwork with Mallory's and Van Gieson's stains. Though the cellular infiltration and tubular degeneration were always patchy, in three rabbits the patches were so large and close together that there was little normal tissue remaining. In the guinea pigs, too, though the lesions never compared in intensity with those in the rabbits, they often assumed a rather diffuse character. In the dogs the focal nature of the lesion was perfectly evident and in the cat where they were least marked there were rarely more than one or two patches in a section.

As a rule, the glomeruli showed little change from normal in the kidneys. There was congestion of the capillary tufts with occasionally a small coagulum in the glomerular space, but in five rabbits, one of which showed no other changes, distinct abnormalities of acute type were seen in the glomeruli. These consisted in swelling and proliferation of the capillary endothelium with occasionally a karyorrhexis of the nuclei of the cells. The capillaries were almost free of red blood cells, but scattered through the loops small numbers of small round cells were observed (figure 9). Not all glomeruli were affected but usually one or two in every microscopic field showed some degree of change, while the most marked alterations occurred in those glomeruli that were situated in the cellular areas.

In what was assumed to be the third stage of this process, the connective tissue increased and glomerular lesions formed the most conspicuous part. It was less frequently encountered than the more acute cellular infiltrations, possibly because most of the ani-

mals were killed before there was time for their development. But these changes were present in six rabbits, one dog, two cats, and three guinea pigs. They consisted in the presence of dense fibrous rays extending from the midzone into the cortex, and, as a rule, following the course of a blood vessel, sometimes to the capsule. Often there was more or less round cell infiltration of the connective tissue together with increase in connective tissue about the arcuate vessels (figures 10 and 11). The strands of connective tissue were not very wide and not numerous, averaging about the thickness of two or three convoluted tubules in cross section, and rarely more than two or three patches were seen to a section. In these areas the tubules were atrophied and contained hyaline casts. Occasionally a more diffuse increase in connective tissue was seen between well preserved tubules.

The glomeruli both in the fibrous rays and sometimes in isolated instances through the cortex showed the most marked departure from normal. In the fibrous patches scarcely any normal glomeruli were found (figure 12). There was marked fibrous thickening of the capsule, which in a few instances appeared as a hyaline ring, and the capillary endothelium showed advanced proliferation, so that the capillary tuft appeared as a mass of cells totally devoid of red blood corpuscles (figure 13). Some glomeruli showed complete hyaline degeneration. In three guinea pigs a few isolated glomeruli were seen which showed a proliferation of the capsular epithelium leading to a characteristic crescent formation, and in a fourth about two thirds of all the glomeruli showed swelling and proliferation of the capsular epithelium with thickening of the capillary tufts.

Though I have described the lesions in the kidneys of these animals as a sequence of events in the development of a nephritis sometimes of a severe grade, it would only be possible to do so from the study of a considerable series of cases, for in an individual instance two of these types of lesions might occur in the same kidney and it was not unusual to see the focal necrosis of tubular epithelium in combination with the more extensive cellular infiltration or even with the advanced connective tissue formation. The appearance of these lesions, too, was not absolutely uniform in sequence, though the production of connective tissue was not observed except in ani-

mals that had been inoculated over a period of several weeks. Two rabbits, for instance, one of which had been subjected to three intravenous intoxicating doses of horse serum and five of egg-albumen, the other to three intravenous intoxicating doses of horse serum and six of egg-albumen, over a period of sixteen days, presented extensive cellular infiltration of the kidney, while the third animal in the series sensitized in the same manner at the same time and given intravenously three intoxicating doses of horse serum and six of egg-white over a period of sixteen days, showed only a few foci of small round cell infiltration in the cortex. As a rule, however, the most extensive and advanced lesions were seen in the animals that had received the greatest number of intoxicating doses over the longest period of time.

Of the six rabbits that showed normal kidneys at autopsy one had received six, another three intoxicating doses of horse serum, one had received three intoxicating doses of horse serum and four of egg-white, while the other three had received egg-white alone, one two, one four, and the third eleven intoxicating doses intravenously.

Of the five cats that showed normal kidneys, one had received three, one six intoxicating doses of horse serum, one three intravenous injections of horse serum and five of egg-white, one four of horse serum and four of egg-white, and one twelve injections of egg-white alone. Of the four dogs that showed normal kidneys at autopsy, one very large dog, weighing 15,000 grams, had received seven doses of egg-white intravenously and one seven inoculations of egg-white.

The following protocols illustrate the characteristic lesions and the phases of the experiments.

PROTOCOLS.

Rabbit 9.—Weight 1,800 gm.
Oct. 21, 1912. Sensitization with 0.1 c.c. horse serum intraperitoneally.
Oct. 23, 1912. Urine clear; no albumin.
Nov. 22, 1912. 5 c.c. horse serum subcutaneously. No symptoms.
Nov. 25, 1912. 4 c.c. horse serum intravenously. No symptoms.
Dec. 4, 1912. 3 c.c. horse serum subcutaneously. No symptoms.
Dec. 28, 1912. 3 c.c. horse serum intravenously. Slight symptoms.
Dec. 30, 1912. Urine dark; heavy trace of albumin.
Jan. 5, 1913. 1 c.c. horse serum intravenously. Slight symptoms.
Jan. 8, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 9, 1913. 12 hour specimen of urine; heavy trace of albumin; no casts.

Jan. 10, 1913. 1 c.c. horse serum intravenously. Slight symptoms.

Jan. 10, 1913. Urine shows trace of albumin; a few hyaline and granular casts.

Jan. 11, 1913. Urine shows heavy trace of albumin; many hyaline and granular casts.

Jan. 13, 1913. 1.5 c.c. horse serum intravenously. Moderate symptoms.

Jan. 15, 1913. 1.5 c.c. horse serum intravenously. Moderate symptoms.

Jan. 17, 1913. 2 c.c. horse serum intravenously. Marked symptoms.

Jan. 20, 1913. 3 c.c. horse serum intravenously. No definite symptoms.

Jan. 22, 1913. 4 c.c. horse serum intravenously. No definite symptoms.

Jan. 24, 1913. Urine pale, cloudy; heavy trace of albumin; many hyaline and granular casts.

Jan. 24, 1913. 5 c.c. horse serum intravenously. No definite symptoms.

Jan. 27, 1913. 10 c.c. horse serum intravenously. Moderate symptoms. Killed at 4 P. M. Weight 1,580 gm.

Autopsy.—Lungs collapsed. Heart's blood fluid. Liver smooth, appearance normal. Spleen small and red. Stomach and intestines normal. Kidneys small, capsule strips readily, surface smooth. On section cortex and medulla not well defined; pale streaks through the papilla.

Microscopical Examination.—Kidneys show degeneration of groups of tubules near the midzone with infiltration of the round cells about them. These areas occur in patches most often about the arcuate vessels, extending thence into the cortex and medulla. Many tubules contain casts. Glomeruli often contain blood but they appear cellular. Endothelium of capillary tufts swollen, and the nuclei often show karyorrhexis. Many small round cells in loops.

Rabbit 15.—Weight 1,415 gm.

Sensitized with the following injections: Dec. 31, 1912, and Jan. 2, 1913. 2 c.c. horse serum intravenously. Jan. 3, 1913. 5.5 c.c. horse serum intravenously. Jan. 6, 1913. 2 c.c. egg-white intravenously. Jan. 7, 1913. 2.5 c.c. egg-white intravenously.

Jan. 15, 1913. Urine very cloudy. No albumin.

Results of injections intended to produce anaphylactic shock.

Jan. 27, Feb. 6, and Feb. 11, 1913. 1 c.c. horse serum intravenously. After each dose very severe symptoms.

Jan. 28, 1913. 0.1 c.c. egg-white intravenously. Very slight symptoms.

Jan. 30, 1913. 0.4 c.c. egg-white intravenously. Very severe symptoms.

Feb. 4, 1913. 0.5 c.c. egg-white intravenously. Moderate symptoms.

Feb. 7, 1913. 0.5 c.c. egg-white intravenously. Very severe symptoms. Urine clear; moderate amount of albumin.

Feb. 11, 1913. Urine clear; large amount of albumin, detritus, many hyaline casts.

Feb. 12, 1913. 0.5 c.c. egg-white intravenously. Very severe symptoms. Death in 27 minutes.

Autopsy.—Blood fluid. Heart and lungs normal. Spleen soft. Liver large, dark brown, showing gray lines on surface. Kidney rather large and pale, capsule strips easily. Surface smooth, mottled gray and red. On section the papilla is greatly swollen, the cortex pale, differentiation between the two is poor, and the whole surface is streaked with gray lines.

Microscopical Examination.—Kidneys show most extensive areas of small round cell infiltration obscuring portions of the cortex and midzone. Necrosis of cells of collecting tubules with extensive surrounding small round cell infiltration. In places delicate strands of connective tissue are seen running into the cortex. Many glomeruli show increase of tuft cells with infiltration of small round cells.

Rabbit 17.—Weight 1,610 gm.

Sensitized with the following injections: Dec. 31, 1912. 3 c.c. horse serum intravenously. Jan. 2, 1913. 2 c.c. horse serum intravenously. Jan. 3, 1913. 5 c.c. horse serum intravenously. Jan. 6, 7, and 9, 1913. 2.5 c.c. egg-white intravenously.

Jan. 15, 1913. Urine slightly turbid; no albumin.

Results of injections intended to produce anaphylactic shock.

Jan. 27, and Feb. 6 and 11, 1913. 1 c.c., 1.5 c.c., and 2 c.c. horse serum intravenously. Slight symptoms following each dose.

Jan. 27, 1913. 0.1 c.c. egg-white intravenously. No definite symptoms.

Jan. 28, 1913. 0.25 c.c. egg-white intravenously. Slight symptoms.

Jan. 30, 1913. 0.5 c.c. egg-white intravenously. Slight symptoms.

Jan. 24, 1913. 0.6 c.c. egg-white intravenously. No definite symptoms.

Feb. 7, 1913. 0.8 c.c. egg-white intravenously. Moderate symptoms.

Feb. 12, 1913. 1 c.c. egg-white intravenously. Death within 2 minutes.

Autopsy.—Heart contracted. Lungs collapsed, blood fluid. Heart and lungs normal. Liver brown, white streaks on surface. Kidney appears normal except for a few depressed points on surface. Section shows an occasional gray streak in the cortex.

Microscopical Examination.—There are a few patches of round cell infiltration between the tubules, with occasional increase in connective tissue.

Rabbit 28.—Weight 1,495 gm. Control for rabbits 25 and 30.

Feb. 25, 27, and 28, 1913. Sensitizing doses of 1, 1.5, and 1.5 c.c. of egg-white intravenously.

Feb. 28, 1913. Killed. All organs appear normal.

Microscopical Examination.—Kidneys normal.

Rabbit 30.—Weight 1,420 gm.

Feb. 25, 27, and 28, 1913. Sensitizing doses. 1.2 and 1.5 c.c. egg-white intravenously, and 2 c.c. egg-white intraperitoneally.

Mar. 7, 1913. Urine (12 hour specimen) cloudy; faint trace albumin; no casts.

Mar. 24, 25, 26, 27, and Apr. 4, 1913. 0.1, 0.2, 0.2, 0.4, and 0.5 c.c. egg-white intravenously, respectively. After 1st and 2d doses severe symptoms; after 3d, 4th, and 5th mild symptoms.

Apr. 8, 1913. Urine (12 hour specimen) shows marked trace of albumin; scattered hyaline and granular casts.

Apr. 8, 12, 14, and 19, 1913. 0.5, 1, 2, and 2 c.c. egg-white intravenously, respectively. After each dose severe symptoms.

Apr. 26, 1913. 1.5 c.c. egg-white intravenously. Very severe symptoms. Death in 48 hours.

Autopsy.—Heart dilated, lungs collapsed and showed reddish patches. Liver showed numerous pin-point sized opaque gray areas. Kidneys pale, medium size, occasional gray streaks from medulla and cortex.

Microscopical Examination.—The kidneys show very few changes in the cortex. Almost all the glomeruli appear abnormal. The capillary tufts contain no blood, and the endothelial cells are swollen, show many karyorrhectic figures, and many small mononuclear cells.

Rabbit 25.—Weight 1,440 gm.

Sensitized with the following injections: Feb. 25 and 27, 1913. 1 and 1.5 c.c. egg-white intravenously, respectively. Feb. 28, 1913. 2.5 c.c. egg-white intraperitoneally. Mar. 3, 4, and 5, 1913. 2.5 c.c. horse serum intravenously.

Mar. 5, 1913. Urine clear; no albumin.

Results of injections intended to produce anaphylactic shock.

Mar. 24 and 25, 1913. 0.1 c.c. egg-white intravenously, marked symptoms.

Mar. 27, 1913. 0.2 c.c. egg-white intravenously; slight symptoms.

Apr. 4 and 8, 1913. 0.5 c.c. egg-white intravenously. Marked and moderate symptoms.

Apr. 14, 1913. 1 c.c. egg-white intravenously, slight symptoms.

Apr. 21 and 28, and May 13, 1913. 2 c.c. egg-white intravenously. Severe to moderate symptoms.

May 19 and 26, 1913. 2.5 c.c. egg-white intravenously. Moderate symptoms.

Mar. 25, 1913. 0.2 c.c. horse serum intravenously. Very severe symptoms.

Apr. 7, 1913. 0.4 c.c. horse serum intravenously. Mild symptoms.

Apr. 12, 1913. 1 c.c. horse serum intravenously. Moderate symptoms.

Apr. 19, 1913. 1.5 c.c. horse serum intravenously. Slight symptoms.

Apr. 26, 1913. 2.5 c.c. horse serum intravenously. Violent symptoms.

May 10, 1913. 2 c.c. horse serum intravenously. Very severe symptoms.

May 15, 1913. 2.5 c.c. horse serum intravenously. Mild symptoms.

May 21, 1913. 4 c.c. horse serum intravenously. Mild symptoms.

May 27, 1913. 8 c.c. horse serum intravenously. Mild symptoms.

June 2, 1913. 3 c.c. egg-white intravenously. Very severe symptoms. Death in 6 minutes.

Autopsy.—Heart contracted, blood fluid, lungs collapsed, normal. Spleen normal. Liver shows slight lobulation. Kidneys soft, swollen, pale, few red dots on surface. On section the papilla is swollen and gray, and shows streaks extending into the cortex which is pale with poorly marked striæ.

Microscopical Examination.—Kidneys show very extensive cellular fibrous streaks extending from midzone to cortex. Here and there are areas of degenerated tubules with surrounding round cell infiltration. Glomeruli show swelling of capillary endothelium.

Rabbit 101.—Weight 1,250 gm.

Sensitized with the following injections: Mar. 18 and 19, 1913. 1.5 c.c. egg-white intravenously. Mar. 20, 1913. 2.5 c.c. egg-white intraperitoneally.

Results of injections intended to produce anaphylactic shock.

Apr. 10, 1913. 1 c.c. egg-white intravenously. Severe symptoms.

Apr. 17, 1913. 1.5 c.c. egg-white intravenously. Very severe symptoms. Death in 5 minutes.

Autopsy.—Heart contracted, lungs collapsed, blood fluid. All organs appear normal, except for extreme congestion.

Microscopical Examination.—Kidneys show marked congestion and cloudy swelling of tubular epithelium.

Guinea Pig 3.—Weight about 400 gm.

Oct. 21, 1912. Sensitizing dose. 0.01 c.c. horse serum intraperitoneally.

Nov. 12, 1912. 2 c.c. horse serum intraperitoneally. No definite symptoms.

Dec. 27, 1912. 1.5 c.c. horse serum intraperitoneally. Mild symptoms.

Jan. 20, 1913. 2 c.c. horse serum intraperitoneally. No symptoms.

Feb. 7, 1913. 3 c.c. horse serum intraperitoneally. Slight symptoms.

Mar. 3, 1913. 2 c.c. horse serum intraperitoneally. No symptoms.

Mar. 3, 1913. 5 c.c. horse serum intraperitoneally. No symptoms.

Mar. 19, 1913. 3 c.c. horse serum intraperitoneally. No symptoms.

Apr. 15, 1913. 4 c.c. horse serum intracardially. Violent symptoms. Died in 7 minutes.

Autopsy.—Lungs insufflated, stand alone. No hemorrhage in any organ. Extreme congestion.

Microscopical Examination.—Kidneys show well marked cellular infiltration of midzone and cortex, extending down into the papilla. Most of the glomeruli show extreme congestion. A few show proliferation of the capsular epithelium, forming typical crescents.

Guinea Pig 8.—Weight about 400 gm.

Oct. 21, 1912. Sensitizing dose. 0.01 c.c. horse serum intraperitoneally.

Nov. 22, 1912. 2 c.c. horse serum intraperitoneally. Moderate symptoms.

Dec. 27, 1912. 2.5 c.c. horse serum intraperitoneally. Marked symptoms.

Jan. 20, 1913. 3 c.c. horse serum intraperitoneally. No symptoms.

Feb. 11, 1913. 4 c.c. horse serum intraperitoneally. Slight symptoms; scratching at nose.

Mar. 3, 1913. 3 c.c. horse serum intraperitoneally. Slight symptoms. 5 c.c. horse serum intraperitoneally. No symptoms.

Apr. 7, 1913. 4 c.c. horse serum intraperitoneally. No symptoms. 5 c.c. horse serum intraperitoneally. No symptoms.

Apr. 21, 1913. 1.5 c.c. horse serum intracardially. Slight symptoms.

May 21, 1913. 2 c.c. horse serum intracardially. Very severe symptoms. Death May 22.

Autopsy.—May 22, 1913. Heart and lungs normal. Stomach enormously distended showing many hemorrhages. Hemorrhage over colon. Spleen large and soft. Liver large, pale, mottled with red. Kidneys intensely congested.

Microscopical Examination.—Kidneys show increase in connective tissue about arcuate vessels with round cell infiltration and occasional thickening of glomerular capsules.

Guinea Pig 1.—Weight 295 gm.

Oct. 18, 1912. Sensitized with 0.5 c.c. egg-white intraperitoneally.

Nov. 11. 2 c.c. egg-white intraperitoneally. Very severe symptoms. Death in 30 minutes.

Autopsy.—Typical emphysema of lungs. Congestion of all organs.

Microscopical Examination.—Kidneys show congestion with acute degeneration of tubular epithelium.

Cat 2.—Weight 3,250 gm.

Nov. 19, 1912. Sensitizing dose. 3 c.c. horse serum subcutaneously.

Dec. 9, 1912. 2 c.c. horse serum intravenously. Very severe symptoms.

Jan. 2, 1913. 2 c.c. horse serum intravenously. Very severe symptoms. 22

minutes later 4 c.c. horse serum intravenously. Very slight symptoms. 17 minutes later 8 c.c. horse serum intravenously. No symptoms.

Jan. 3, 1913. Urine (12 hour specimen) clear; albumen ¼ of 0.1 per cent.; no casts.

Jan. 16, 1913. 2.75 c.c. horse serum intravenously. Very severe symptoms. Death in 7 minutes.

Autopsy.—Blood fluid. Lungs collapsed. Heart contracted. Liver and spleen congested. Kidney pale, fatty.

Microscopical Examination.—Kidneys show congestion, much fat in tubular epithelium. No change in glomeruli nor interstitial tissues.

Cat 4.—Weight 3,510 gm.

Nov. 19, 1912. Sensitized with 3 c.c. horse serum subcutaneously.

Dec. 9, 1912. 1 c.c. horse serum intravenously. Severe symptoms.

Jan. 2, 1913. 8 c.c. horse serum intraperitoneally. No definite symptoms. 25 minutes later 11.5 c.c. horse serum intraperitoneally. No symptoms.

Jan. 16, 1913. 10 c.c. horse serum intraperitoneally. No immediate symptoms. Diarrhea.

Jan. 27, 1913. 6 c.c. horse serum intraperitoneally. Moderate symptoms.

Feb. 11, 1913. 8 c.c. horse serum intraperitoneally. Mild symptoms.

Feb. 24, 1913. 6 c.c. horse serum intraperitoneally. Mild symptoms. 30 minutes later 10 c.c. horse serum intraperitoneally. No symptoms.

Mar. 12, 1913. 5 c.c. horse serum intraperitoneally. No definite symptoms. 12 minutes later 3.5 c.c. horse serum intraperitoneally. Severe symptoms. 8 minutes later 3 c.c. horse serum intraperitoneally. Slight symptoms.

Mar. 27, 1913. 5.5 c.c. horse serum intraperitoneally. Moderate symptoms.

Apr. 12, 1913. 6.5 c.c. horse serum intraperitoneally. No symptoms.

Apr. 29, 1913. 7 c.c. horse serum intraperitoneally. No symptoms.

May 28, 1913. 8.5 c.c. horse serum intraperitoneally. No symptoms.

June 11, 1913. Killed. Has lost weight, looks badly. Weight 2,400 gm. Omentum and retroperitoneal tissues thickened and opaque. Mesenteric and retroperitoneal lymph nodes large and soft. Heart and lungs normal. Kidneys large, red, and show on section a few whitish streaks.

Microscopical Examination.—Kidneys show much thickening of connective tissue about arcuate vessels, with considerable round cell infiltration. There are connective tissue striæ that extend into the cortex. In these areas tubules are atrophied. There is thickening of glomerular capsules with hyaline degeneration of capillary tufts. There are also a few isolated areas of round cell infiltration beneath the capsules.

Cat 8.—Weight 3,095 gm.

Mar. 12, 1913. Sensitizing dose. 2 c.c. egg-white intravenously.

Apr. 8, 1913. 0.4 c.c. egg-white intravenously. Mild symptoms. 25 minutes later 0.8 c.c. egg-white intravenously. No symptoms.

Apr. 19, 1913. 1 c.c. egg-white intravenously. Severe symptoms. After last dose cat continued ill.

Apr. 23, 1913. Killed.

Autopsy.—Heart shows a few hemorrhages over the left auricle. Lungs normal. Liver large, soft, mottled gray and red. Kidneys appear normal.

Microscopical Examination.—Kidneys show swelling of tubular epithelium

and occasionally cellular streaks extending along blood vessels from the midzone out towards the capsule. Some of the glomeruli in these areas show thickening of the capsule.

Dog 4.—Weight 6,890 gm.

Nov. 7, 1912. Urine by catheter clear; faintest trace of albumin; no casts.

Nov. 7, 1912. Sensitizing dose of 5 c.c. horse serum subcutaneously.

Nov. 27, 1912. 12.5 c.c. horse serum subcutaneously. Moderate symptoms.

Dec. 30, 1912. 2.25 c.c. horse serum intravenously. Very slight symptoms. 15 minutes later 3 c.c. horse serum intravenously. Very slight symptoms. 25 minutes later 3.5 c.c. horse serum intravenously. No symptoms. 10 minutes later 5 c.c. horse serum intravenously. No symptoms. 40 minutes later 8 c.c. horse serum intravenously. Vomiting and diarrhea. 25 minutes later 10 c.c. horse serum intravenously. Continued vomiting.

Dec. 31, 1912. Urine (24 hour specimen) 650 c.c., clear; faint trace of albumin; no casts.

Jan. 15, 1913. 2.5 c.c. horse serum intravenously. Mild symptoms.

Jan. 22, 1913. 4 c.c. horse serum intravenously. No definite symptoms.

Jan. 29, 1913. 10 c.c. horse serum intravenously. Mild symptoms.

Jan. 30, 1913. Killed.

Autopsy.—Liver and kidneys rather pale, but all organs appear normal.

Microscopical Examination.—Throughout the midzone of the kidneys there is a streaky cellular infiltration, extending into the cortex and occasionally into the papilla. It is not very marked. No changes in the glomeruli.

Dog 1.—Brindle. Weight 6,060 gm.

Sensitized with the following injections: Oct. 25, 1912. 5 c.c. horse serum subcutaneously. Jan. 6, 1913. 5 c.c. egg-white intravenously.

Oct. 29, 1912. Urine clear, amber; faintest trace of albumin; no casts.

Nov. 22, 1912. 10 c.c. horse serum subcutaneously. Moderately severe symptoms.

Nov. 23, 1912. Urine heavy trace albumin.

Dec. 9, 1912. 10 c.c. horse serum subcutaneously. Mild symptoms.

Dec. 10, 1912. Urine faint trace albumin.

Dec. 11, 1912. Urine heavy trace albumin.

Jan. 6, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 7, 1913. 1.1 c.c. horse serum intravenously. No symptoms.

Jan. 8, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 9, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 10, 1913. 1 c.c. horse serum intravenously. Much scratching.

Jan. 11, 1913. 2 c.c. horse serum intravenously. Moderately severe symptoms.

Jan. 13, 1913. 1 c.c. horse serum intravenously. No symptoms.

Jan. 15, 1913. 1.5 c.c. horse serum intravenously. No symptoms.

Jan. 16, 1913. 2 c.c. horse serum intravenously. No symptoms.

Jan. 17, 1913. 4 c.c. horse serum intravenously. No symptoms.

Jan. 18, 1913. 5 c.c. horse serum intravenously. No symptoms.

Jan. 20, 1913. 7 c.c. horse serum intravenously. No symptoms.

Jan. 24, 1913. 8.5 c.c. horse serum intravenously. No symptoms.

Jan. 27, 1913. 0.5 c.c. egg-white intravenously. No symptoms.

Jan. 28, 1913. 2.5 c.c. egg-white intravenously. No symptoms.

Jan. 31, 1913. Urine (over night) clear; large amount albumin.

Jan. 31, 1913. 2.5 c.c. egg-white intravenously. No symptoms.

Feb. 4, 1913. 5 c.c. egg-white intravenously. Severe symptoms. Lost considerable weight; looks badly. Weight 5,310 gm.

Feb. 6, 1913. Killed.

Autopsy.—Heart, lungs, spleen, and liver normal. Kidneys dark, capsule adherent, surface slightly irregular. On section the medulla is large and pale, the arcuate vessels are well marked with white streaks extending from the medulla into the cortex.

Microscopical Examination.—Marked cellular infiltration of the papilla and midzones of the kidney, with increase in connective tissue about the vessels and rays of cellular connective tissue extending into the cortex. Glomeruli do not show any marked changes.

Dog 9.—Fox terrier type. Control. Weight 3,200 gm.

Mar. 3, 1913. 3 c.c. horse serum subcutaneously.

Mar. 3, 1913. 2 c.c. egg-white subcutaneously.

Six days later developed pneumonia and died.

Autopsy.—Mar. 10, 1913. Lungs show extensive consolidation. Other organs show cloudy swelling and congestion.

Microscopical Examination.—Kidneys show cloudy swelling of tubular epithelium. No other changes.

Dog 10.—Fox terrier type. Weight 4,360 gm.

Mar. 3, 1913. Sensitizing dose. 3 c.c. horse serum intravenously.

Mar. 3, 1913. Sensitizing dose. 2 c.c. egg-white intravenously.

Mar. 24, 1913. 1 c.c. egg-white intravenously. Slight symptoms.

Apr. 4, 1913. 3 c.c. egg-white intravenously. Very severe symptoms. Death in 24 minutes.

Autopsy.—Blood fluid. Lungs collapsed, heart normal, liver large, dark, bloody. Kidneys regular, pale and gray. On section the medulla and cortex show a few gray lines and streaks.

Microscopical Examination.—Infiltration of small round cells in the midzone, papilla, and cortex. Congestion of glomeruli, some of which appear very cellular.

The nephritis that has appeared in these animals seems to be very much like that which Dickson[26] describes as resulting from the injection of uranium nitrate, though most of the animals in my experiments did not show as extensive lesions as Dickson pictures. The changes in the epithelial structures of the kidney that I have observed point to the fact that there is some direct toxic action from the repeated injections of horse serum and egg-white upon the epithelium, particularly of the cells lining the loops of Henle and the collecting tubules. This is followed or perhaps accompanied by an infiltration of small round cells producing the histological appearance of a chronic inflammation, and leading later to new connective

[26] Dickson, E. C., *Arch. Int. Med.*, 1909, iii, 375; 1912, ix, 557.

tissue formation. It is probable that these round cells come from the circulating blood and not from the fixed connective tissue cells of the kidney, for in a number of sections the capillaries surrounding small foci of necrosis were seen to be filled with small mononuclear cells.

Besides the necrosis of the epithelium there is a direct injury to the capillary tufts of the glomeruli, for the acute changes described were often seen in glomeruli far distant from any areas of tubular degeneration and atrophy and occasionally in kidneys that presented no other marked abnormality. Some of the more advanced glomerular changes in the areas where tubules were atrophied and where there was much increase in interstitial tissue might be considered as secondary to the destruction of the tubule unit, but in many instances, when such factors were lacking, there must have been primary injury to the glomerulus.

It is known that injection of uranium nitrate may produce chronic nephritis in animals, and recently Baehr[27] and Christian[28] have shown that with the same drug true glomerular lesions may be obtained in rabbits. Ophüls[29] has described a chronic nephritis as developing in rabbits intoxicated over long periods with lead, and consequently it is not so much the possibility of producing in animals a chronic or subacute nephritis that I should like to emphasize as the method of bringing about this lesion through repeated injections of foreign protein.

In all these experiments it is necessary to keep in mind the fact that laboratory animals and especially rabbits and dogs are occasionally subject to a spontaneous nephritis which might lead to a grave error in interpreting the results. With a view to determining the frequency of spontaneous nephritis in animals, Ophüls[30] has examined the kidneys of fifty supposedly normal rabbits and one hundred guinea pigs. Many of these were old animals. Of the fifty rabbits, twenty-eight had normal kidneys, nine showed slight parenchymatous lesions, three a few small areas of cellular infiltration, and ten well marked increase in the interstitial tissue. Changes

[27] Baehr, G., *Beitr. z. path. Anat. u. z. allg. Path.*, 1913, lv, 545.
[28] Christian, H. A., and O'Hare, J. P., *Jour. Med. Research*, 1913, xxviii, 227.
[29] Ophüls, W., *Jour. Med. Research*, 1908, xviii, 497.
[30] Ophüls, W., *Proc. Soc. Exper. Biol. and Med.*, 1910–11, viii, 75.

in the glomeruli were never seen. Of the guinea pigs, sixty-three had absolutely normal kidneys, and thirty-five showed foci of small round cell infiltration through the cortex. In a few instances the glomerular capsules were thickened. I have examined as a control the kidneys of twenty-four supposedly normal rabbits. The kidneys in sixteen were absolutely normal, in four they showed well marked old fibrous scars in the cortex, which did not resemble the lesions in the experiments that I have described, and in four there were one or two patches of small round cells between the tubules of the cortex which were not unlike the isolated areas of round cell infiltration seen in some of my experiments. In no instance were glomerular lesions encountered. The kidneys of fifteen cats, some taken directly from the street, others kept for several weeks in the laboratory, have also been examined, and with one exception, proved normal. The kidneys of this cat appeared coarsely granular and microscopically showed old connective tissue scars running through the cortex. There were no glomerular lesions. In my experiments I have made every attempt to exclude the possibility of using animals that might have spontaneous nephritis. Young animals were always employed. The rabbits usually weighed between 1,000 and 1,500 grams, and were selected from different lots. The guinea pigs weighed from 200 to 400 grams. An attempt was also made to use young dogs, but the ages of the cats were not known.

Before the inoculations an examination of the urine for albumin and casts was made and often repeated two or three times. If the urine contained more than the faintest trace of albumin, or cells or casts on centrifugalization, the animal was discarded. During the inoculations, as may be seen from some of the protocols, the urine of many animals showed considerable amounts of albumin with great numbers of casts, both hyaline and granular. This was most marked during the few days following an inoculation.[31] Occasionally great numbers of epithelial cells and mononuclear cells were seen, especially in dogs.

Finally, to exclude even more definitely the possibility that I might be dealing with spontaneous lesions, three dogs sensitized to

[31] When egg-white was employed, the albumin was always discountenanced, since egg-albumin after parenteral inoculations is eliminated unchanged in the urine.

egg-white and horse serum were subjected to operation with the assistance of Dr. Burnap, and a portion of one kidney of each dog was excised for control examination before the secondary series of inoculations was begun. The microscopical examination of this resected portion showed that at operation the kidneys of all three dogs were normal. Twenty-six days after the primary inoculations and twenty days after operation these dogs were subjected to intravenous injections of egg-white and horse serum.

Dog 12 received two doses of horse serum and three of egg-white. There were no marked symptoms after the injections of horse serum. Severe symptoms developed after the second and third injections of egg-white, and the dog died within twenty-four hours after the last injection. During these injections, the urine, which before had been normal, showed increasing amounts of albumin with epithelial cells, mononuclear cells, and granular casts. At autopsy the kidneys were rather large and pale. Microscopical examination showed edema of the interstitial tissue with scattered round cells particularly in the papilla and intermediate zone. There was extensive degeneration of the epithelium of Henle's loops and of the collecting tubules. Through the cortex areas of extensive degeneration involving the convoluted tubules were seen and the lumina of many tubules contained granular casts.

Dog 13 received four doses of egg-white and four of horse serum intravenously. During this period the urine, which before had been normal, showed traces of albumin, great numbers of epithelial cells, and small round cells and later many hyaline and granular casts. After each dose of foreign protein there were severe symptoms. He was killed thirty-four days after the first injection. The kidneys at autopsy were rather large and showed grayish markings in the intermediate zone. Microscopically all sections showed patches of degeneration of the tubular epithelium in the cortex surrounded by small round cells with diffuse small round cell infiltration of the intermediate zone.

Dog 14 received one injection of horse serum and two of egg-white, dying acutely from the second injection. At autopsy the kidneys were congested, and on microscopical examination showed extensive diffuse degenerative changes in the tubular epithelium

with a few foci of small round cell infiltration that in one instance surrounded many glomeruli (figure 14).

These three experiments demonstrate definitely that sensitized dogs whose kidneys are normal may, within a few weeks, develop nephritis from repeated injections of small amounts of horse serum and egg-white. On account of the constancy of the lesions described in the kidneys in the various animals, therefore, the involvement of the glomeruli, the development of urinary signs of nephritis, as well as the three experiments just described, it is justifiable to exclude spontaneous changes as a cause of the nephritis occurring in these animals and to conclude that it is a direct result of the injections of foreign protein.

A more difficult question is to determine definitely whether the lesions in the kidney really depend upon a sensitization of the animals to foreign protein and represent an effect of anaphylaxis, or whether the foreign protein itself, irrespective of sensitization, acts as a toxin, such as uranium nitrate, and brings about the nephritis in the same way that a chemical irritant or poison might. Though horse serum is almost harmless for rabbits, guinea pigs, and dogs, Schultz[32] states that it may produce grave symptoms or death in young cats when injected intravenously in doses of 0.0025 of a cubic centimeter per gram of body weight. Doses of this size, which would mean six to eight cubic centimeters for the cats employed were rarely given intravenously in my experiments. Moreover, the possibility that the serum might be eliminated as such through the kidneys and thus cause some irritating effect or injury is highly improbable. Though there has been some discussion as to the possibility of an animal's utilizing the protein of foreign serum injected intravenously, Lommel's[33] statement that the injection of horse serum in dogs increases the total nitrogen output in the urine 50 to 90 per cent., is not upheld by the recent experiments of Austin and Eisenbrey[34] which show that as much as eighty cubic centimeters of horse serum may be injected intravenously in dogs which are in nitrogen equilibrium without the appearance of albumin

[32] Schultz, W. H., *loc. cit.*

[33] Lommel, F., *Arch. f. exper. Path. u. Therap.*, 1907–08, lviii, 50.

[34] Austin, J. H., and Eisenbrey, A. B., *Arch. Int. Med.*, 1912, x, 305.

or increased incoagulable nitrogen in the urine. I have not observed an albuminuria in normal animals following the injection of horse serum in the doses used in the experiments. With egg-white, however, the results were different. This substance is in itself toxic to rabbits when injected in large doses, and Vaughan[35] has shown that repeated small doses may cause fever and other untoward symptoms. Egg-white, unlike horse serum, when introduced parenterally is eliminated unchanged through the kidneys, as was first shown by Stokvis.[36] Among others, Friedemann and Isaac[37] and Van Alstyne and Grant[38] have studied this question and conclude that native protein as well as the egg albumin is eliminated under these circumstances. That large quantities of albumin appear in the urine of dogs, cats, and rabbits very shortly after an intravenous injection of egg-white, I have observed repeatedly, and though no accurate investigations were made as to the exact time of appearance, albumin was in a few instances found in the urine within thirty minutes of the intravenous injection of egg-white. The following experiment shows definitely that the albumin found in the urine was in part egg-albumin.

Dog 5.—Weight 15,000 gm. Urine containing much albumin was collected for eighteen hours after injection of egg-white intravenously, and filtered through a Berkefeld filter. 3 c.c. of this filtered urine were injected intraperitoneally into each of two guinea pigs, 1 and 2, weighing 220 and 235 gm.; at the same time 0.5 c.c. of pure egg-white were injected intraperitoneally into guinea pig 3, weighing 222 gm.

After 3 weeks:

Guinea pig 1 was given 2.5 c.c. of egg-white intraperitoneally. Death in 15 min.
Guinea pig 2 was given 1.5 c.c. of egg-white intraperitoneally. Death in 5 min.
Guinea pig 3 was given 2.5 c.c. of egg-white intraperitoneally. Death in 4 min.
Normal control 4 was given 2.5 c.c. of egg-white intraperitoneally. No symptoms.

It is evident that under the conditions of these experiments egg-albumin was eliminated as such by the kidneys, and it is possible that the passage of this substance through the cells of the kidney might in itself cause some injury.

[35] Vaughan, V. C., *loc. cit.*

[36] Stokvis, J. B., *Centralbl. f. d. med. Wissensch.*, 1864, ii, 596.

[37] Friedemann and Isaac, S., *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 513; 1906, iii, 209.

[38] Van Alstyne, E. V. N., and Grant, P. A., *Jour. Med. Research*, 1912, xxv, 399.

In order to investigate this matter I have injected a certain number of normal animals with single doses or closely spaced doses of horse serum or egg-white or both. One dog which had received two cubic centimeters of egg-white intravenously and five cubic centimeters of horse serum subcutaneously and was killed five days later at the beginning of an attack of distemper, showed a few patches of small round cells in the cortex. Another dog injected in the same manner showed normal kidneys. None of the four sensitized guinea pigs dying acutely from the first dose of protein showed chronic lesions. Of ten rabbits, three inoculated with two to four consecutive doses of egg-white, one with casein, and six with three consecutive doses of egg-white and three of horse serum, five showed distinct changes in the kidneys after three to sixteen weeks. The most marked lesions were seen in three rabbits that received one dose of 1.5 cubic centimeters of egg-white intravenously, one of 2.5 cubic centimeters intraperitoneally, and one of 5 cubic centimeters subcutaneously on three successive days, and one week later two successive intravenous doses of 2.5 cubic centimeters of horse serum. These animals all received excessive doses and though the lesions did not compare in severity with many of the experimental series, it is evident that under these circumstances, particularly when one of the doses is very large and given subcutaneously, nephritis may develop. It will be necessary, however, to investigate this point further before any very definite conclusions may be drawn as regards the primary toxicity of egg-white for the kidneys of laboratory animals.

In the majority of experiments, however, advanced nephritis was obtained only after the frequent repetition of injections of small doses of egg-white or horse serum in sensitized animals, and though it is impossible at the present time to say definitely that the development of nephritis depends exclusively upon the previous sensitization of the animal to these substances, it seems probable that at least any primary toxicity of the protein for the kidney cells was heightened by this procedure, and that the lesions in the kidney as well as those in the liver, myocardium, and peritoneum are to be interpreted as a generalized reaction analogous to the local Arthus phenomenon in the skin of the rabbit.

CONCLUSIONS.

The repeated injection of small doses of horse serum and egg-white in dogs, cats, rabbits, and guinea pigs that have been sensitized to these proteins, causes injury to the cells of various organs and tissues with resulting inflammatory reactions.

The changes are especially marked after intraperitoneal injections in the peritoneum and after intravenous injections in the livers of rabbits and cats, and in the myocardium and kidneys of all groups of animals.

In dogs and rabbits, especially, there develops a well marked nephritis characterized by degeneration and necrosis of the epithelium of the loops of Henle, of the collecting tubules, and less frequently of the convoluted tubules. This is accompanied by an extensive small round cell infiltration of the interstitial tissue and later the formation of connective tissue. Together with these changes there are acute and chronic alterations in the glomeruli of all groups of animals.

Egg-white in large doses is itself injurious to the kidney of animals, but this slight primary toxicity is probably greatly enhanced through previous sensitization of the animal.

EXPLANATION OF PLATES.

PLATE 56.

FIG. 1. Rabbit 16. Fibrous thickening and round cell infiltration of the omentum, after three intraperitoneal injections of horse serum and six intraperitoneal injections of egg-white in a sensitized animal.

FIG. 2. Rabbit 16. New formation of cellular connective tissue of portal spaces of liver. Note the number of cells in the capillaries of the peripheral zone, after three injections of horse serum and six of egg-white in a sensitized animal.

PLATE 57.

FIG. 3. Rabbit 24. Necrosis and round cell infiltration of the myocardium of the right ventricle, after twelve injections of egg-white in a sensitized animal.

PLATE 58.

FIG. 4. Rabbit 21. Area in the papilla of a kidney showing early necrosis and desquamation of the cells of the collecting tubules, after ten injections of egg-white in a sensitized animal.

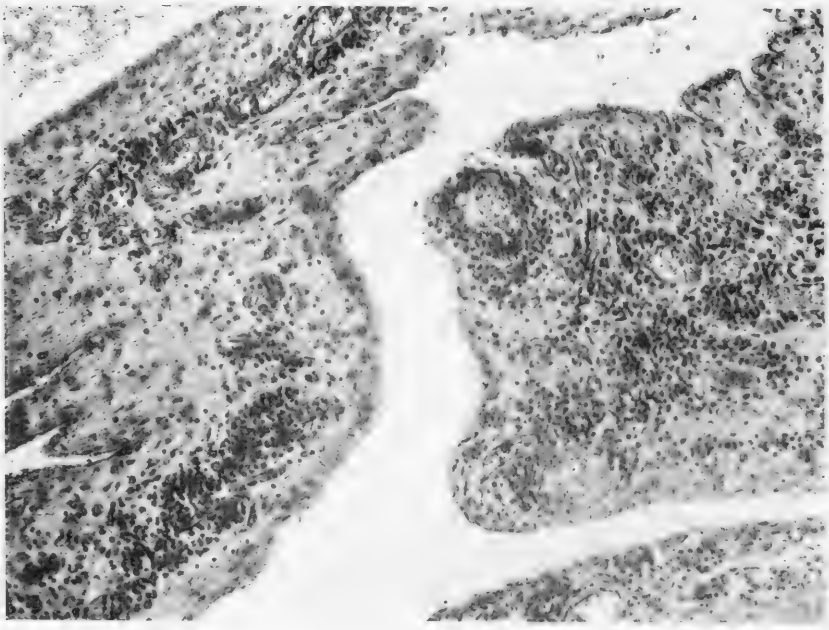

FIG. 1.

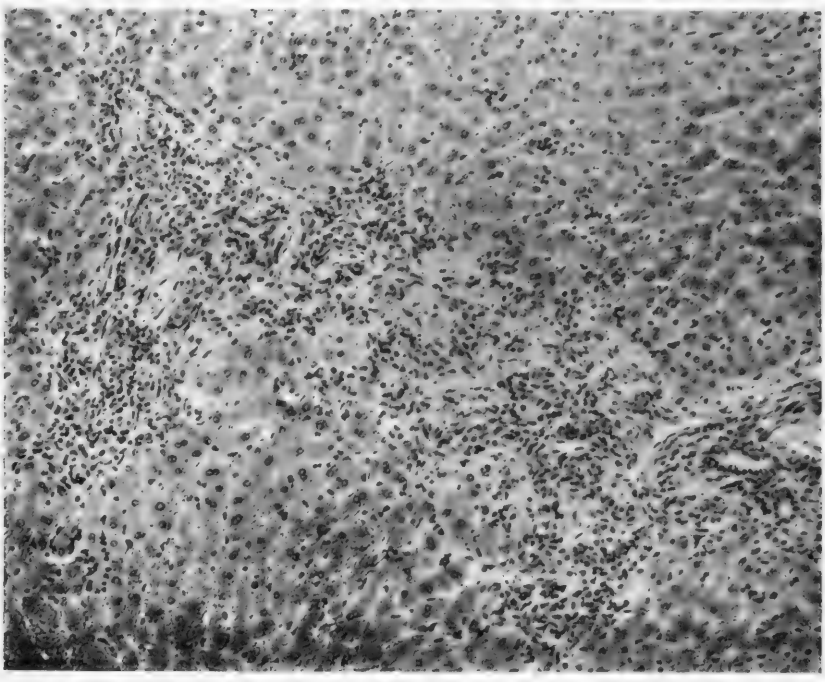

FIG. 2.

(Longcope: Production of Experimental Nephritis.)

CIHM

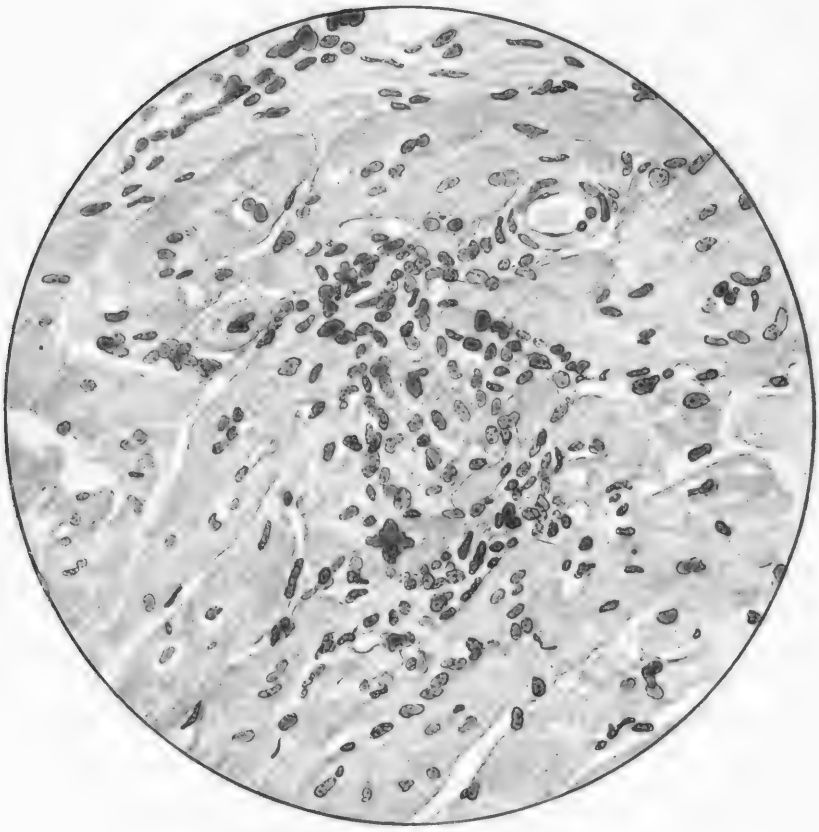

FIG. 3.

(Longcope: Production of Experimental Nephritis.)

FIG. 4.

(Longcope: Production of Experimental Nephritis.)

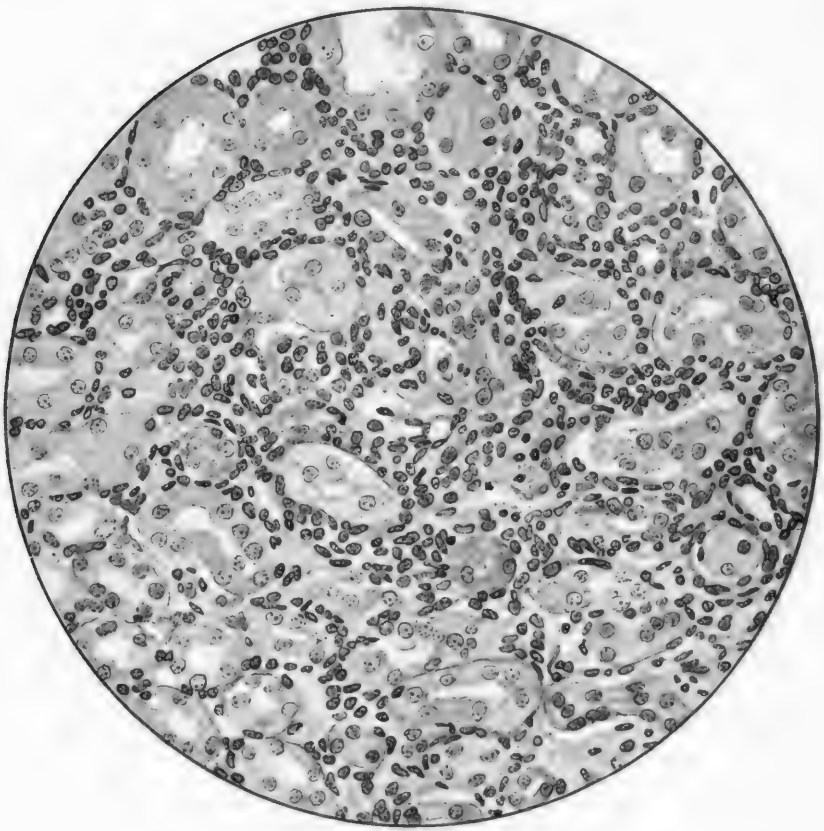

FIG. 5.

(Longcope: Production of Experimental Nephritis.)

FIG. 6.

(Longcope: Production of Experimental Nephritis.)

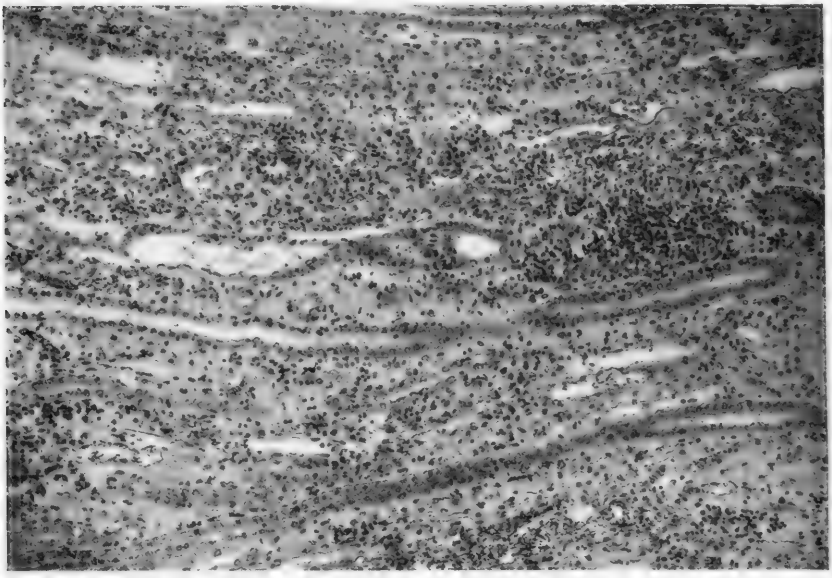

FIG. 7.

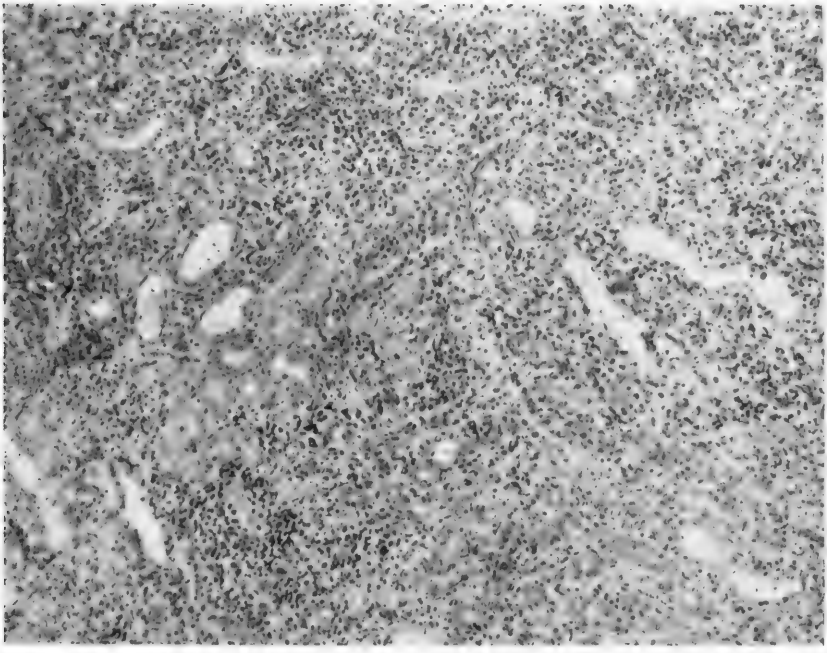

FIG. 8.

(Longcope: Production of Experimental Nephritis.)

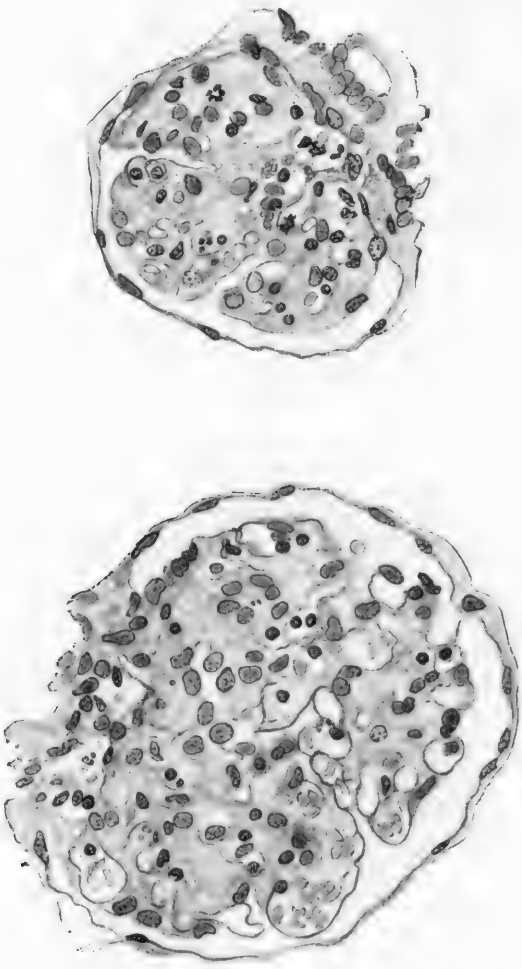

FIG. 9.

(Longcope: Production of Experimental Nephritis)

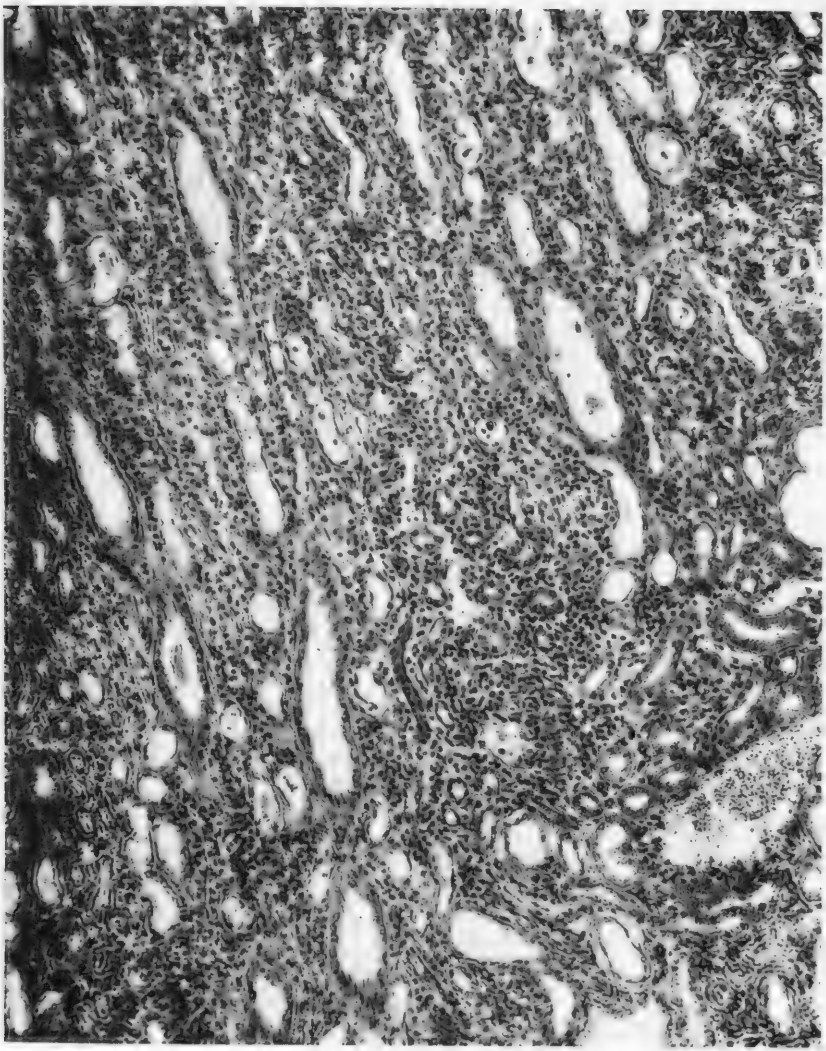

FIG. 10.

(Longcope: Production of Experimental Nephritis.)

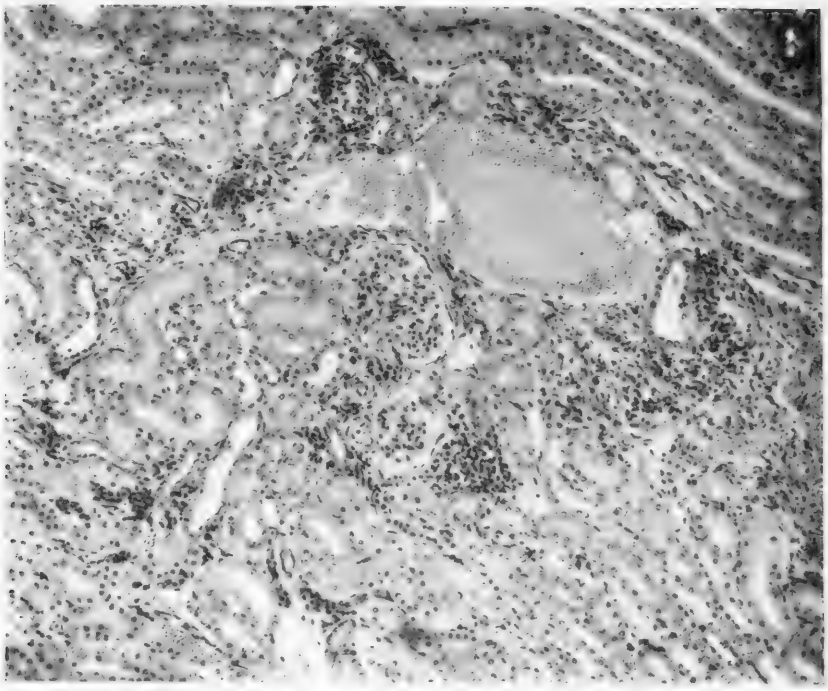

FIG. 11.

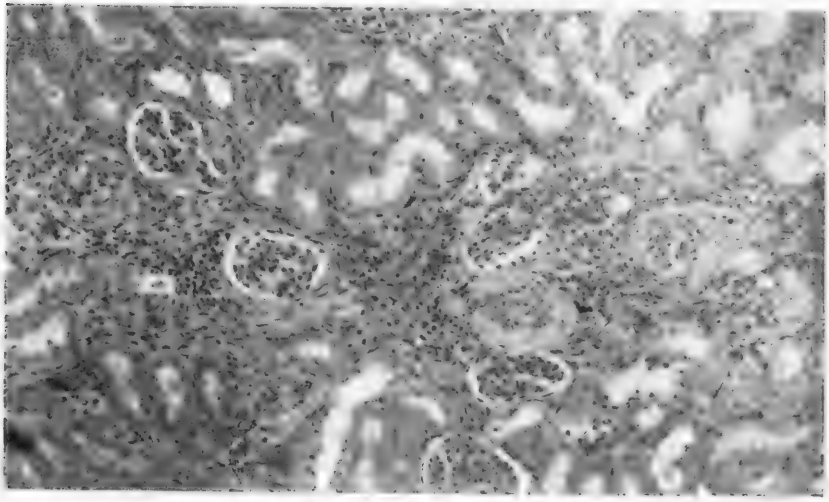

FIG. 12.

(Longcope: Production of Experimental Nephritis.)

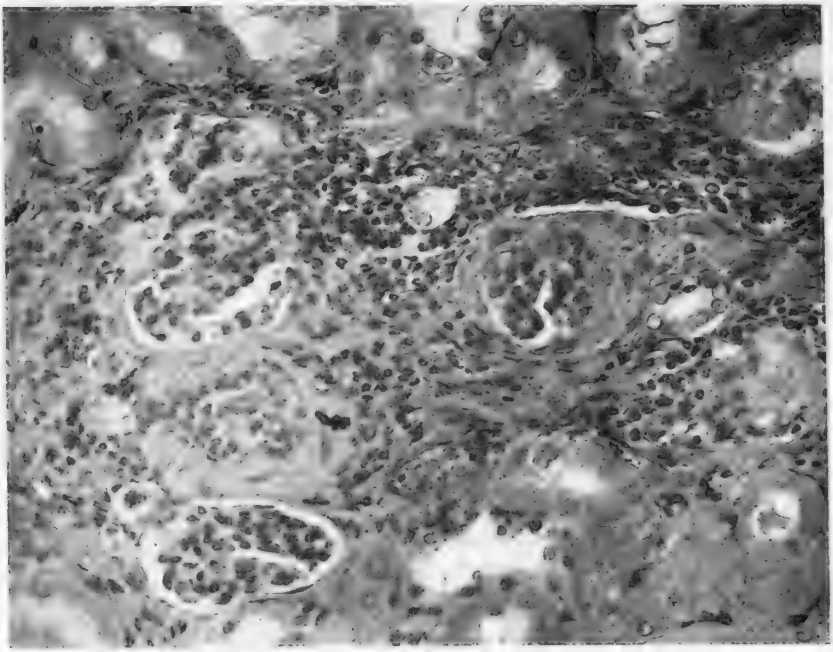

FIG. 31.

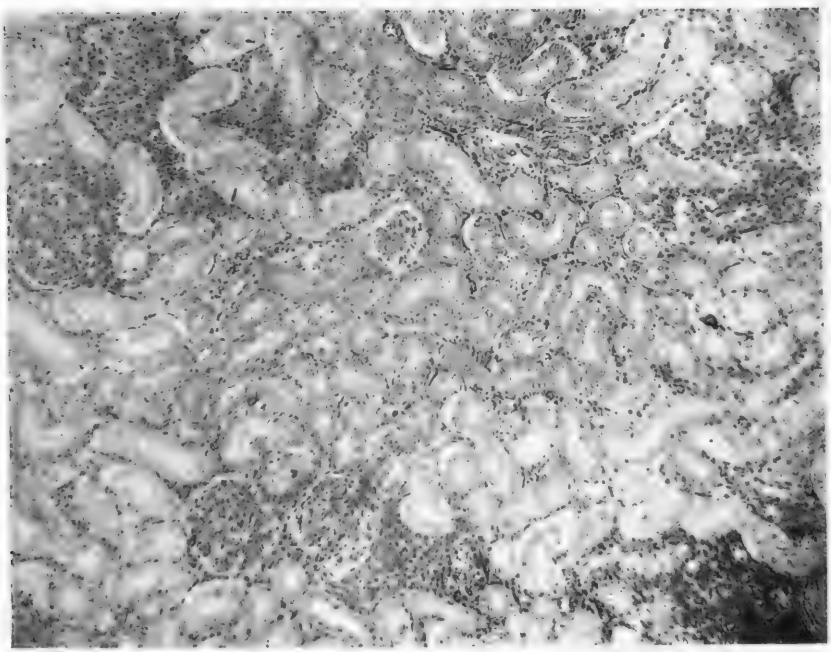

FIG. 14.

(Longcope: Production of Experimental Nephritis.)

PLATE 59.

FIG. 5. Rabbit 9. Early necrosis of the tubular epithelium of the kidney with round cell infiltration, after fourteen injections of horse serum in a sensitized animal.

PLATE 60.

FIG. 6. Rabbit 15. Intermediate zone and cortex of the kidney showing extensive round cell infiltration, degeneration of the tubular epithelium, and early connective tissue formation, after three injections of horse serum and five of egg-white in a sensitized animal.

PLATE 61.

FIG. 7. Rabbit 15. Area in the papilla of the kidney showing necrosis of the epithelium of the convoluted tubules with round cell infiltration.

FIG. 8. Rabbit 15. Another area in the cortex of the kidney.

PLATE 62.

FIG. 9. Rabbit 25. Glomeruli showing swelling of the endothelial cells, infiltration by mononuclear cells and karyorrhexis of nuclei, after seven injections of horse serum and ten of egg-white in a sensitized animal.

PLATE 63.

FIG. 10. Rabbit 16. Area of round cell infiltration and connective tissue formation in the intermediate zone of the kidney, after three inoculations of horse serum and six of egg-white intraperitoneally in a sensitized animal.

PLATE 64.

FIG. 11. Dog 4. Area of connective tissue increase and round cell infiltration in the intermediate zone of the kidney, after thirteen intravenous injections of horse serum and four intravenous injections of egg-white in a sensitized animal.

FIG. 12. Cortex of rabbit 5 showing fibrous area with extensive involvement of the glomeruli, after eleven injections of horse serum over a period of 130 days.

PLATE 65.

FIG. 13. Area in the cortex of rabbit 5, showing the same change as figure 12.

FIG. 14. Dog 14. Cortex of kidney showing round cell infiltration, especially about the glomeruli, after three intravenous injections of egg-white in a sensitized animal. A portion of kidney, removed at operation before injection, was normal.

THE RELATION OF THE AURICULAR ACTIVITY FOLLOWING FARADIZATION OF THE DOG'S AURICLE TO ABNORMAL AURICULAR ACTIVITY IN MAN.*

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PLATES 66 TO 68.

Auricular fibrillation has come to be generally accepted as responsible for a group of phenomena which is frequently met with in patients suffering from heart disease. When the ventricular contractions occur irregularly, when the venous tracings fail to give evidence of definite auricular contractions, but the so called ventricular form of venous pulse curve is obtained, and when electrocardiograms show small, irregular undulations between the irregularly occurring ventricular complexes instead of well defined P waves, fibrillation is considered to have replaced the normal auricular contractions. This widely accepted theory rests largely upon the fact that the electrocardiograms from such patients resemble closely those obtained from dogs after the auricles have been thrown into a state of so called fibrillation. Although Cushny and Edmunds (1) first suggested that a type of irregularity of the heart in man might be dependent on auricular fibrillation, it was not until Lewis (2) and Rothberger and Winterberg (3) compared independently the curves obtained from dogs after auricular faradization with those from patients with cardiac arhythmia, that this dependence could be considered as well established. The auricular activity of the dogs was observed directly while electrocardiograms were obtained from them, and as the curves from the patients resembled closely those from the dogs the form of auricular activity in

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the patients was considered to be the same as that seen in the dogs.

In the present communication it is proposed to consider the question of the identity of the auricular activity following faradization of the dog's auricle and the abnormal auricular activity which occurs in man. In a series of experiments an attempt was made to determine by direct observation the exact nature of the auricular activity of the dog following faradization, and these observations were correlated with electrocardiograms and with myocardiographic curves from the auricles. By noting the type of auricular activity which yielded electrocardiograms most closely resembling those obtained from patients with arhythmia, conclusions seem justified as to the nature of the auricular activity which is present in these patients. Variation in the type of auricular movements was obtained by stimulating the peripheral ends of the vagi, as has been described elsewhere (4).

The auricular activity following faradization of the mammalian auricles has been variously described. McWilliam (5) says that it "differs in various respects from that seen in the ventricles. The application of the current sets the auricles into a rapid flutter, the rapidity of which largely depends upon the excitability of the auricular tissue and the strength of the current employed. The movements are regular,—they seem to consist of a series of contractions originating in the stimulated area and thence spreading over the rest of the tissue. The movement does not show any distinct sign of incoördination,—it looks like a rapid series of contraction waves passing over the auricular walls." Much at variance with this description is that given by Rothberger and Winterberg (3) in their first paper on the relation between arhythmia in man and auricular fibrillation. They described the phenomenon as similar to that seen in the fibrillating ventricles as follows: In place of the strong contractions flashing through the entire auricular musculature at one time, there appears a continuous fine muscular activity, the vibrations and waves of which remind one of a surface of water moved lightly by the wind. This activity can perhaps be best illustrated by recalling to mind the picture of the tongue showing similar fibrillary movements in cases of bulbar paralysis.

Other descriptions of the auricular activity following faradization usually lie between the two foregoing extremes. Winterberg (6) in an earlier publication states that true fibrillation of a general character similar to that seen in the ventricles seldom occurs in the faradized auricles, but contractions involving larger masses of muscle usually occur at the same time as true fibrillatory movements. He considers that the incoördination of the auricular activity can be made out by direct observation without great difficulty. Lewis (2) has observed different grades of fibrillation varying as to the extent of auricular movements, while Hirschfelder (7) considers that either tachycardia or fibrillation may result from auricular faradization, depending upon the

strength of the current used. The observations reported by Rothberger and Winterberg (8) in their second paper concerning auricular fibrillation agree in regard to different grades of fibrillation with those of Lewis, but they describe finally as *"unreines Schlagen"* the simultaneous occurrence of coördinated auricular contractions and true fibrillation following faradization of the dog's auricle.

Our own observations of the activity of the faradized auricles were made on twenty-three dogs. The details of the methods employed are described in a previous communication (4). It may be said, however, that the faradic current was obtained from an inductorium with the secondary coil usually drawn out sixty millimeters, the primary coil of which was attached to a two volt dry cell. The current was applied to the right auricle by means of small clips fastened to the auricular muscle about five millimeters apart. Variations in the strength of the current beyond a certain point had no apparent effect on the character of the tumultuous auricular activity which it set up, although currents apparently too weak to inaugurate this type of activity caused extrasystoles and tachycardia. In eight experiments in which the auricles always resumed the normal rhythmical activity shortly after the end of faradization, changes in the strength of current seemed to have no influence on the time elapsing between the end of faradization and the return of the normal rhythm, the so called *Nachflimmern.* In fifteen experiments the abnormal auricular activity continued from five minutes to over an hour after faradization had ceased, and it was these experiments especially which gave good opportunities for studying directly the character of the auricular activity.

Under the condition of our experiments this tumultuous auricular activity could be seen to consist of very rapid movements, apparently contractions of the whole auricles, quite similar to those described by McWilliam (5). But besides these rapid contractions, fine fibrillatory movements in the various muscle fibers could be seen. When the peripheral end of the right vagus was stimulated, the general contractions ceased, the auricles, as a whole, remained stationary, and the fine fibrillatory movements like those seen in fibrillating ventricles persisted. At this time the auricles had an appearance exactly as described by Rothberger and Winterberg (3), but at no other time was such an appearance observed. When the

stimulation of the nerve was removed, the coarser movements could be seen definitely to begin again slowly, coëxisting with, but not replacing the fine true fibrillatory movements. It is evident, therefore, that the altered activity of the auricles resulting from faradization was not true fibrillation alone, but consisted of two different forms of cardiac activity occurring simultaneously in the auricles, namely, tachycardia and fibrillation.

The electrocardiograms which were usually obtained from the dogs after faradization of the auricles show no evidence of normal auricular activity, but there are, between the irregularly recurring ventricular complexes, many waves varying in shape, size, and occurrence. These waves vary in height, not only in different animals, but in records from the same animal, and they are frequently four or five millimeters high. This indicates the passage of a current of four tenths to half a millivolt, as the string of the galvanometer was adjusted so that a deviation of one centimeter in the curve represents a passage of one millivolt (figure 1). At times the auricular activity produced much larger irregular waves in the electrocardiograms (figure 2). These irregular waves of various sizes usually occurred at a rate averaging 500 to 600 per minute. The larger waves were often interrupted by much smaller, finer waves. This auricular activity gave definite waves in records obtained with the Roy and Adami myocardiograph. As this instrument was attached to the auricles so that the auricular waves in the records represented true muscular shortening and lengthening of the whole auricles, it is evident that definite contractions occurred (figure 11 of previous paper (4)). During right vagus stimulation, when true fibrillation alone was seen in the auricles, the myocardiograph yielded no waves in the record (figure 12 of previous paper (4)). At this time the irregular waves of auricular activity in the electrocardiograms were also definitely altered. They became smaller, more irregular, and less distinct. Often during right vagus stimulation, these waves practically disappeared for short intervals (figure 3) (see also figures 5 and 12 of previous paper (4)). As it was seen that true auricular fibrillation existed only during right vagus stimulation and before the auricles had recovered from the effect of faradization, it is only the curves obtained under these

conditions that can be considered as records of true auricular fibrillation.

The electrocardiograms obtained from patients with the form of cardiac arhythmia which has been attributed to auricular fibrillation are familiar to all who have followed clinical electrocardiography. Examples are given in figures 4 to 6. It is seen that there is no evidence of normal auricular activity, but waves of varying distinctness occur between the irregularly occurring ventricular complexes which are evidently the waves of abnormal auricular activity. Figures 4 and 5 have been selected from a large number of such curves because they show these waves with unusual distinctness, while figure 6 shows these waves with about the average distinctness.

The electrocardiograms from these patients vary considerably from patient to patient, but they are seldom identical with those obtained from dogs after auricular faradization. The difference was seen when the records of our experiments were compared with curves which we have obtained from many patients with cardiac arhythmia. This difference is also seen in the curves published by Lewis and by Rothberger and Winterberg. The curves published by the former observer show that this difference is not caused by opening the chest of the experimental animal, as the type of curves was not changed when the chest wall was intact. In the electrocardiograms from the dog after auricular faradization, the waves of auricular activity, although quite irregular, are usually much larger than those in curves from patients, and this comparison indicates that there is a definite difference between the auricular activity following faradization of the dog's auricle and that occurring in patients with cardiac arhythmia.

A much closer resemblance exists between the electrocardiograms from these patients, however, and electrocardiograms obtained from dogs during right vagus stimulation before the effect of auricular faradization has disappeared, when true fibrillation alone was present in the auricles (figure 3). The conclusion seems justified, therefore, that in these patients with cardiac arhythmia, true fibrillation alone is usually present in the auricles.

A form of abnormal auricular activity has been recently de-

scribed in man which yields electrocardiograms presenting some features resembling those observed in records from dogs after auricular faradization. This abnormality of the human heart, to which Jolly and Ritchie (9) have given the name "auricular flutter," consists of an auricular tachycardia of high rate, associated with various degrees of partial or complete auriculoventricular dissociation. The auricular rate is usually between 250 and 330 beats per minute, and the ventricular rate is usually rapid and regular. Irregularities may be caused, however, by changes in the degree of dissociation, and render the diagnosis of the condition almost impossible without electrocardiograms. A number of cases of this condition have been recently recognized, and it appears to be a not infrequent cardiac abnormality, which clinically deserves to be considered as an entity. The electrocardiograms which have been published by Jolly and Ritchie (9), Rihl (10), Lewis (11), and Ritchie (12) show rapidly and regularly recurring waves of auricular activity, differing in form from the normal P wave. The interpretation of the electrocardiograms, that auricular tachycardia is present, seems justified. Except when the records indicate that the ventricles are beating independently on account of complete dissociation, a definite relation exists between the auricular waves and the ventricular complexes. This relationship indicates that the ventricles are receiving impulses from definite auricular contractions.

The term "auricular flutter" was apparently suggested to Jolly and Ritchie by McWilliam's description (5) of the auricular activity following faradization of the dog's auricle. From their study of the electrocardiograms from their case, the conception was evidently gained that the cardiac activity resembled that described by McWilliam, as produced in the auricles by faradization. When they experimented with the dog's heart, however, faradizing the auricles after the ventricles had ceased to beat, they concluded that the activity in the dog's auricles resembled that of auricular fibrillation in man and was distinct from the auricular action in their case of auricular flutter. In a later report by Ritchie the comparison to the auricular activity described by McWilliam is again made, and in discussing the cause of auricular flutter he considers the possibility of a localized inflammatory lesion in the auricular wall as a

cause of increased excitability of the auricles comparable to faradization. Hewlett (13) has also compared the auricular activity in a case which was probably auricular flutter to the activity of the faradized dog's auricle. The electrocardiograms which were usually obtained in our experiments after auricular faradization, such as seen in figures 1 and 2, are not identical with those obtained from patients with the so called auricular flutter. In the records from the experiments, where complete heart-block was obviously not present, the large waves of auricular activity usually bear no definite relationship to the ventricular complexes, nor do they have the characteristic regularity of form which seems to represent a true auricular tachycardia undisturbed by any other cardiac activity, as is seen in the records from the cases of flutter. Nor do ventricular complexes in the usual records from the dogs recur with the regularity which is usually present in the records from these patients. From a comparison, then, of the published electrocardiograms from these cases with those usually obtained from our experiments, it is evident that the cardiac activity, which usually followed auricular faradization, was not the same as that which occurs in patients with auricular flutter. This abnormal auricular activity following faradization which can be seen in dogs to consist of true fibrillation and auricular tachycardia, stands, therefore, between auricular flutter, in which auricular tachycardia alone is present and true fibrillation, which is apparently present in the patients with cardiac arhythmia.

Electrocardiograms were recently obtained from a patient which indicate that fibrillation and flutter alternated with each other several times, and during these changes the electrocardiograms resemble closely those obtained from dogs when the abnormal auricular activity caused by faradization is unaffected by vagus stimulation. The curve shown in figure 7 was obtained at 2.47 P. M. In the first part of the curve the ventricular complexes recur at regular intervals, at a rate of 95 per minute, and there are three waves of auricular activity occurring regularly before and one occurring synchronously with each ventricular complex. Thus an electrocardiogram typical of auricular flutter is seen in the first part of the curve to the point*. After this point the waves of auricular ac-

tivity and the ventricular complexes become irregular and no definite correlation can be made out between the auricular and ventricular portions of the curve. This lack of correlation is present in the record obtained ten minutes later, at 2.57 P. M. (figure 8), while the next record, obtained at 3.02 P. M. (figure 9), is a typical electrocardiogram of auricular fibrillation. The ventricular complexes occur at a rate of 120 per minute, and the larger waves of auricular tachycardia are no longer present, but are replaced by indefinite irregular undulations quite similar to those obtained from the dog during right vagus stimulation (figure 3). It seems probable, therefore, that in this patient the auricular tachycardia, the so called auricular flutter, was disturbed during the taking of the record in figure 7 by the development simultaneously of fibrillation in the auricles, and the two types of auricular activity coëxisted for a time, as they can be seen to do in the dog. Finally, when the third electrocardiogram was obtained, fibrillation dominated the auricles, a change having taken place in the record similar to that which occurs in those from dogs when the right vagus is stimulated following auricular faradization. The apparent lack of coördination between the auricular tachycardia and the ventricular activity, which appeared while the curve in figure 7 was being obtained and which is considered to be caused by the development of fibrillation in the rapidly beating auricle, can be seen in electrocardiograms from one case published by Lewis (11) (case 2). This case yielded electrocardiograms which indicate apparent incoördination existing between the rapidly beating auricles and the ventricles. Lewis interprets these curves (figures 23 and 24) as representing a mixture of 3:1 and 2:1 heart-block. The relation of the waves of auricular and ventricular activity is so inconstant, however, even in ventricular cycles where the same degree of block is supposed to exist, that this explanation seems difficult to accept. The ventricular complexes occur irregularly, a fact noted by Lewis, and the type of arhythmia is such that it can hardly be accounted for by the mechanism Lewis suggests, as in figure 24, second lead, the ventricular cycles are of the following duration in terms of 1/30 of a second: 17.5, 14.0, 15.2, 17.0, 18.0, 13.6, 14.0, 13.5, 14.0, and 14.0. The fact that curves of true fibrillation were obtained five days later adds weight to the belief that these electrocardiograms repre-

sent an auricular activity similar to that in our case. It seems probable that in Lewis's case, true fibrillation was present coincidently with auricular tachycardia and served to interfere with the proper coördination of auricles and ventricles. Several of the cases of auricular flutter that have been observed by means of electrocardiograms have been found later to have passed into true auricular fibrillation, and this change seemed to be especially liable after digitalis had been administered.

Changes not unlike those observed in the electrocardiograms of our patient may take place spontaneously in those obtained from dogs after auricular faradization. In nine of the twenty-three experiments the electrocardiograms indicated at times that the auricles were beating rapidly and regularly, well defined P waves regular in form and rhythm and apparently coördinated with the ventricular complexes appearing. In other words, the records indicate that the usual auricular activity which followed auricular faradization became replaced for a time by a cardiac mechanism similar to that seen in cases of auricular flutter. Such an occurrence is seen in figure 10, *A* and *B*. The figure represents one continuous curve and is divided only for purposes of reproduction. The curve shows first the normal sequential beat, going over into the irregular form when the auricles were faradized. The irregular auricular waves become much less marked for a few beats, resembling those of true fibrillation, and then finally definite auricular waves occur, one between each ventricular complex and one evidently coincident with it. These auricular waves bear a constant relation to the ventricular complexes, the P-R time being 0.13 to 0.14 of a second. The ventricular complexes occur with almost perfect regularity at a rate of 212, while the rate of the auricular waves is 424 per minute. Both vagi were tied and cut four minutes after this curve was obtained, and one minute later the electrocardiogram shown in figure 1 was obtained. As this is typical of the usual auricular activity following faradization, it is seen that the auricular tachycardia was again disturbed by the coëxisting fibrillation. This same change occurred in other experiments uninfluenced by the vagi, as it was seen to occur in several experiments in which the vagi had been already cut.

Careful observations of the auricles were not made at times when the records indicate the existence of tachycardia alone. During

stimulation of the left vagus nerve the auricular waves appeared with increased distinctness and regularity, as is seen in curves previously published (figures 6 and 9 of previous paper (4)). Direct observation of the auricles was made during left vagus stimulation, but no appreciable change in the auricular activity could be made out. Left vagus stimulation causes the electrocardiograms to resemble more closely those obtained from cases of auricular flutter, while right vagus stimulation causes them to resemble more closely electrocardiograms from cases of auricular fibrillation. The fact that the auricular activity of the dog may apparently pass spontaneously from one form to the other indicates that these abnormal types of auricular activity are probably closely allied, whether met with under experimental conditions or in patients with cardiac disorder. Lewis and Schleiter (14) have observed the replacement of the usual auricular activity following faradization by tachycardia and use this fact as evidence for the correlation of auricular tachycardia and auricular fibrillation.

SUMMARY AND CONCLUSIONS.

The tumultuous auricular activity which follows faradization of the auricles of mammals and which has been variously described, could be distinctly seen to consist almost constantly in our experiments on dogs of true fibrillatory movements of the separate muscle fibers coëxisting with a rapid auricular tachycardia. During peripheral stimulation of the right vagus nerve the true fibrillation alone existed, the tachycardia being inhibited.

A comparison of the electrocardiograms from dogs with this abnormal auricular activity with those from patients with the type of cardiac arhythmia which has been attributed to auricular fibrillation, and from patients with so called auricular flutter, indicates that the auricular activity in patients with either of these conditions differs somewhat from that usually seen in the faradized auricles of the dog in our experiments. The auricular activity of the cases of cardiac arhythmia is apparently true fibrillation, similar to that seen in the faradized auricles of the dog during right vagus stimulation.

The electrocardiograms from cases of so called auricular flutter usually give no evidence of auricular fibrillation, and the auricular

activity seems to consist of tachycardia alone. Fibrillation may apparently coëxist with the tachycardia in some cases, when the auricular activity seems to resemble closely that usually seen in the dog after auricular faradization. During peripheral stimulation of the left vagus nerve, the electrocardiograms obtained after auricular faradization show changes which render them more nearly similar to those obtained from patients with auricular flutter. The facts that the auricular activity of the faradized auricles of the dog may apparently pass spontaneously into that closely resembling auricular flutter in man, that it may be changed into true fibrillation by right vagus stimulation, and that the abnormal auricular activity in man passes from a state of flutter to that of fibrillation in a similar manner, may be taken as evidence for the belief that auricular fibrillation and auricular flutter in man are closely allied cardiac disorders.

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EXPLANATION OF PLATES.[1]

PLATE 66

FIG. 1. Experiment 3. From a dog five minutes after auricular faradization. Vagi cut.

FIG. 2. Experiment 18. From a dog one minute after auricular faradization.

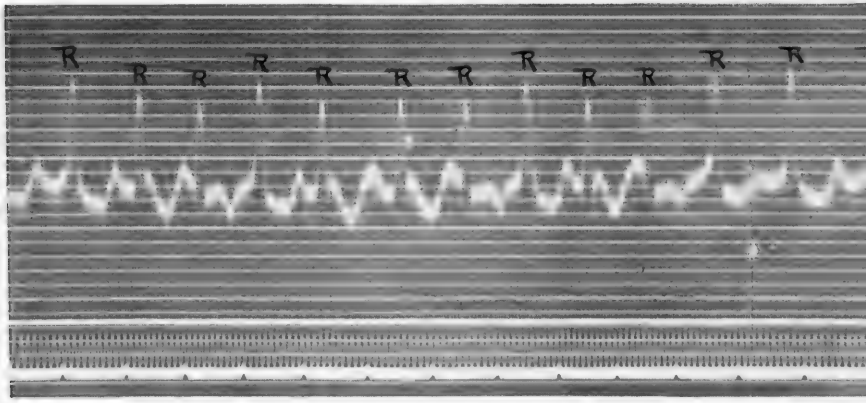
FIG. 3. Experiment 11. From a dog during right vagus stimulation of 5.6 seconds' duration. Thirty seconds after auricular faradization.

FIG. 4. From a patient with cardiac arhythmia of auricular fibrillation. Third lead (left arm and left leg).

FIG. 5. From a patient with cardiac arhythmia of auricular fibrillation. Third lead (right arm and left leg).

FIG. 6. From a patient with cardiac arhythmia of auricular fibrillation. Second lead.

[1] The time marking is in $\frac{1}{50}$ and in $\frac{1}{5}$ of a second.



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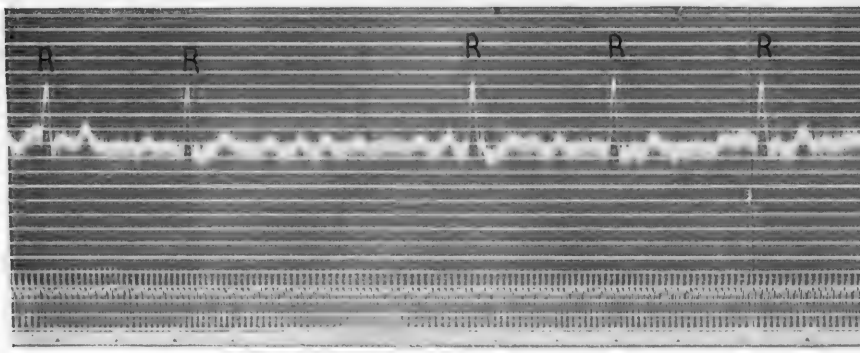


FIG. 3.

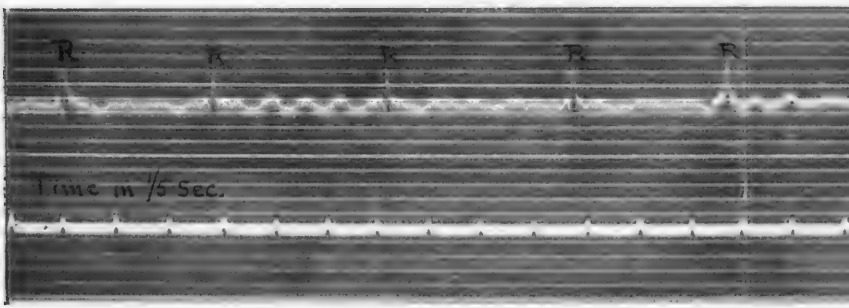
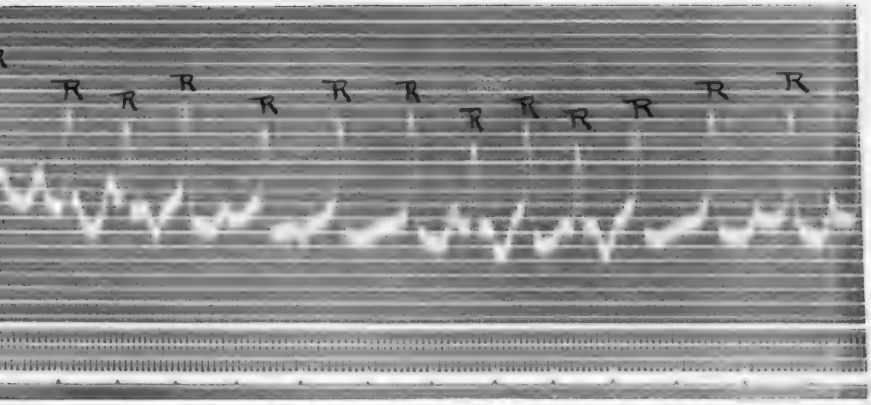


FIG. 5.



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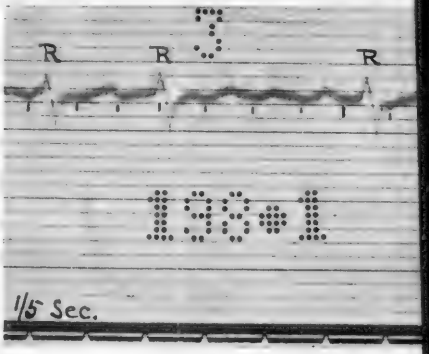
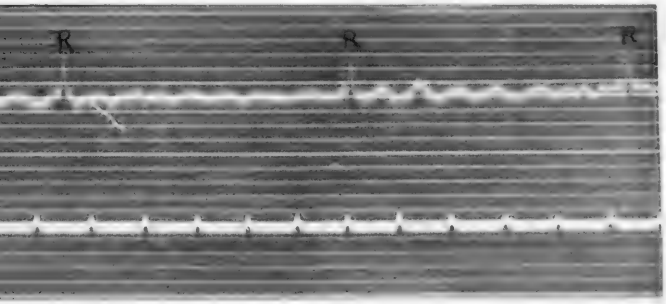
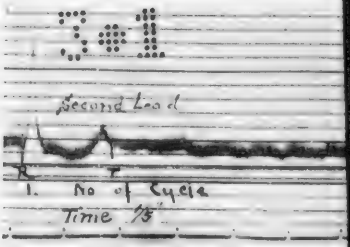






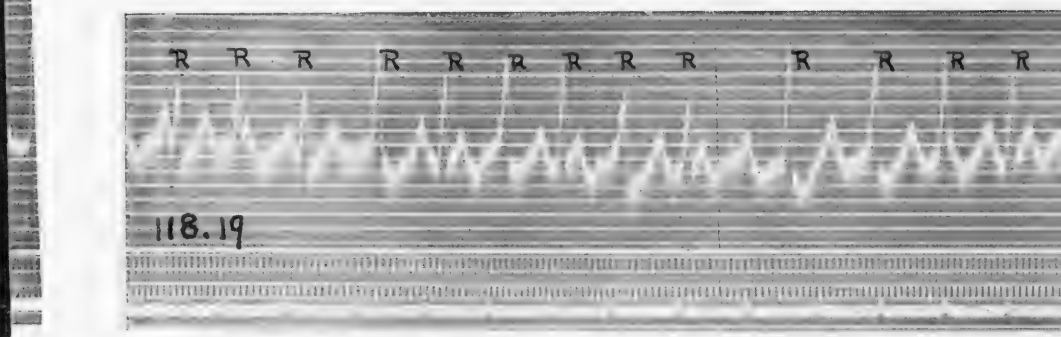


FIG. 2.

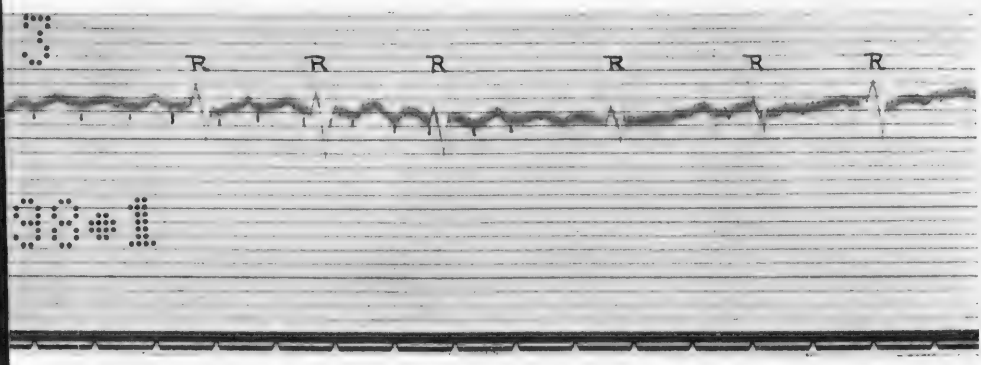


FIG. 4.

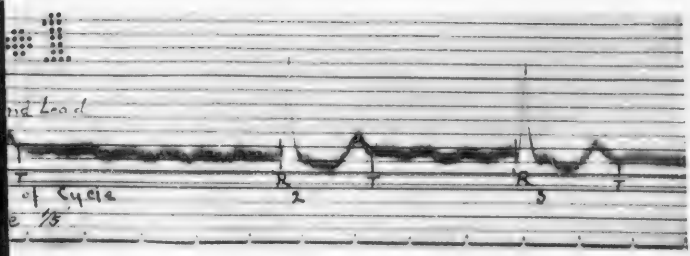
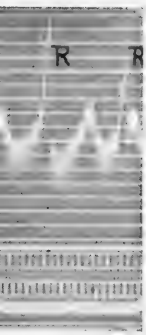


FIG. 6.

(Robinson

PLATE 66.



Auricular Activity)

PLATE 67.

FIGS. 7, 8, and 9. From a patient showing the so called auricular flutter, passing over into cardiac arhythmia of auricular fibrillation.

Figure 7 was obtained at 2.47 P. M.

Figure 8 was obtained at 2.57 P. M.

Figure 9 was obtained at 3.02 P. M.

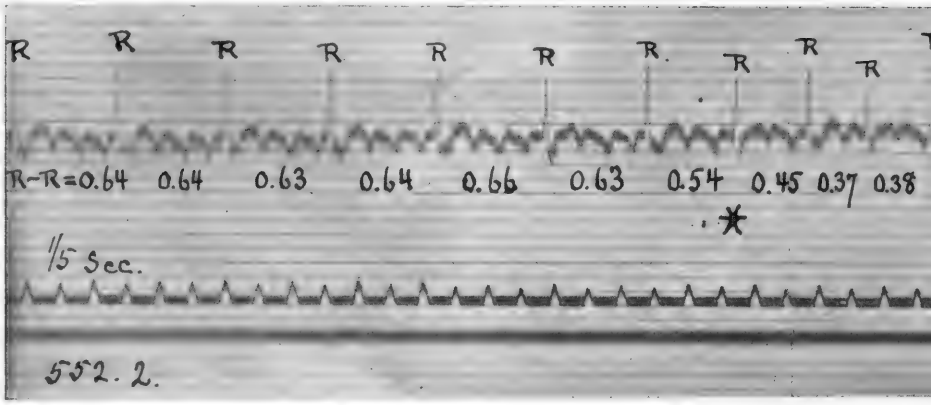


FIG. 7.

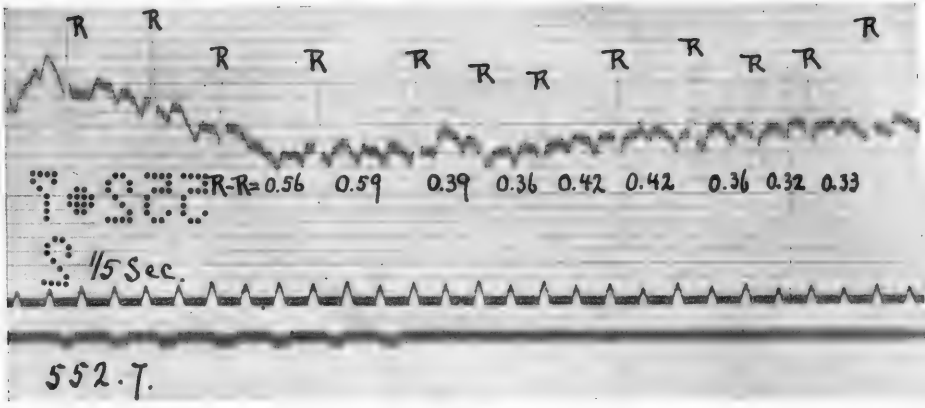


FIG. 8.

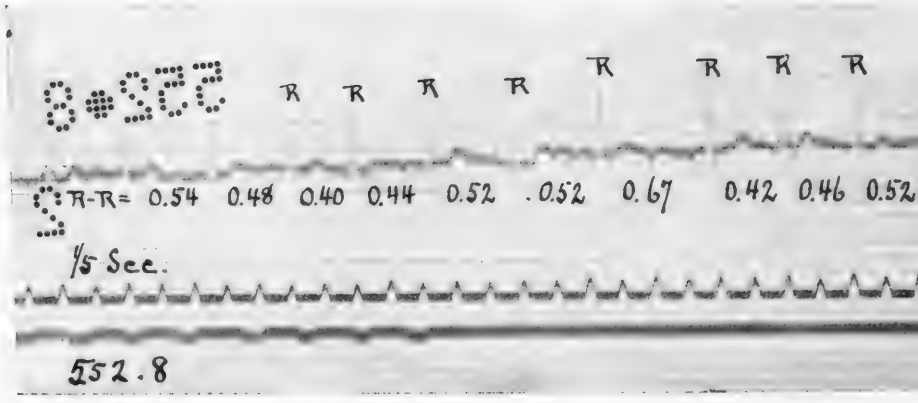
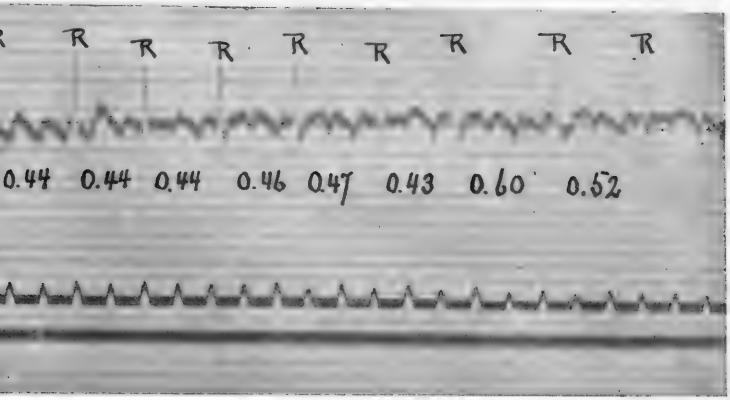
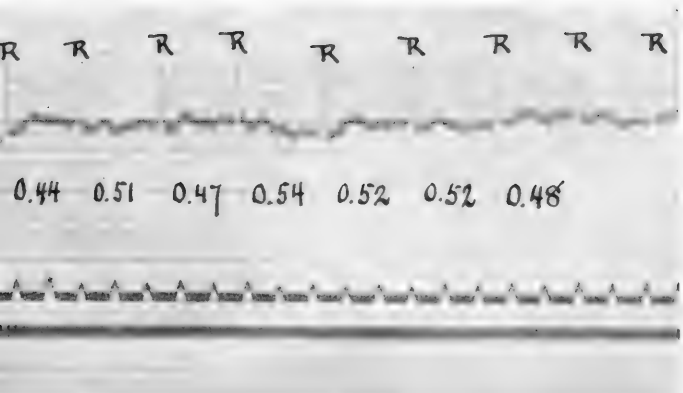


FIG. 9.

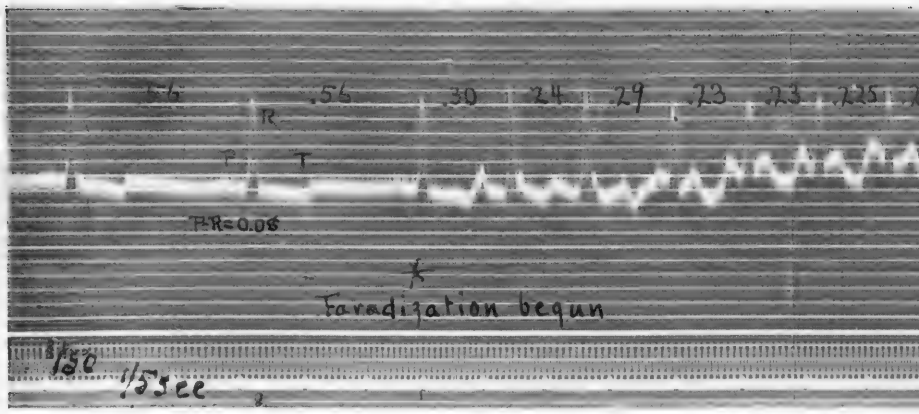


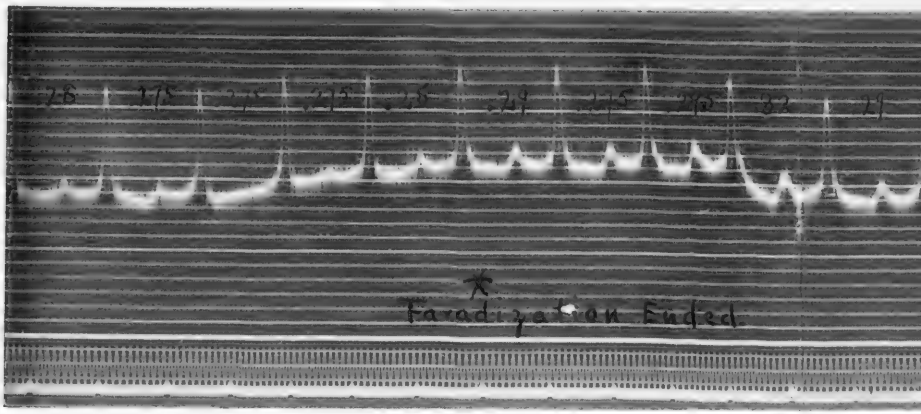


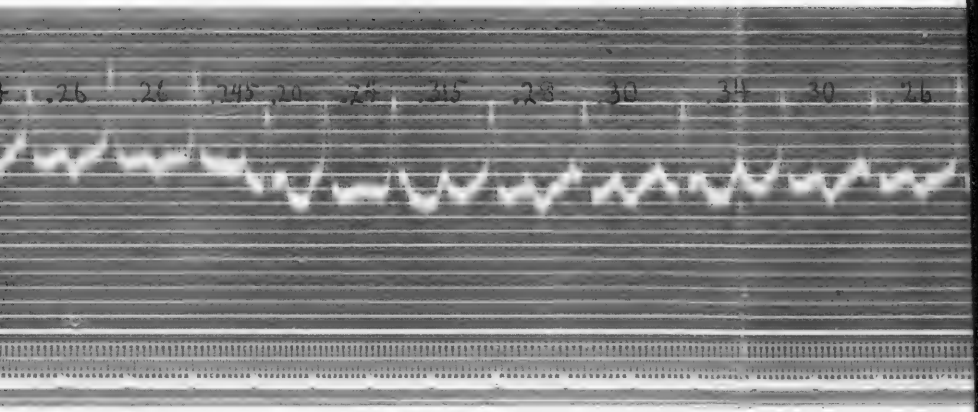
(Robinson: Auricular Activity.)

PLATE 68.

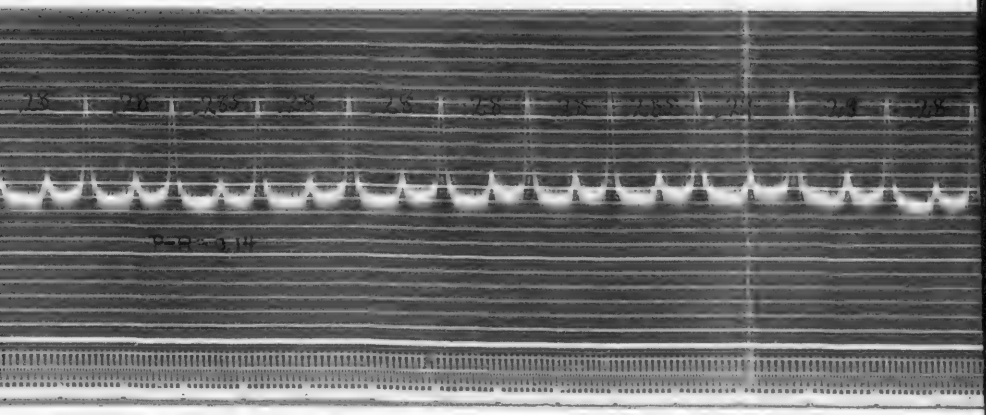
FIG. 10, *A* and *B*. Experiment 3. From the same dog from which the curve in figure 1 was obtained, before, during, and after auricular faradization. The curve was obtained five minutes before that in figure 1. Parts *A* and *B* are continuous.


.56
R
.56
.30
.24
.29
.23
.23
.225
P
T
T-R=0.08
Faradization begun
1/50
1/5 sec


.26
.275
.24
.25
.24
.25
.30
.32
.21
Faradization Ended



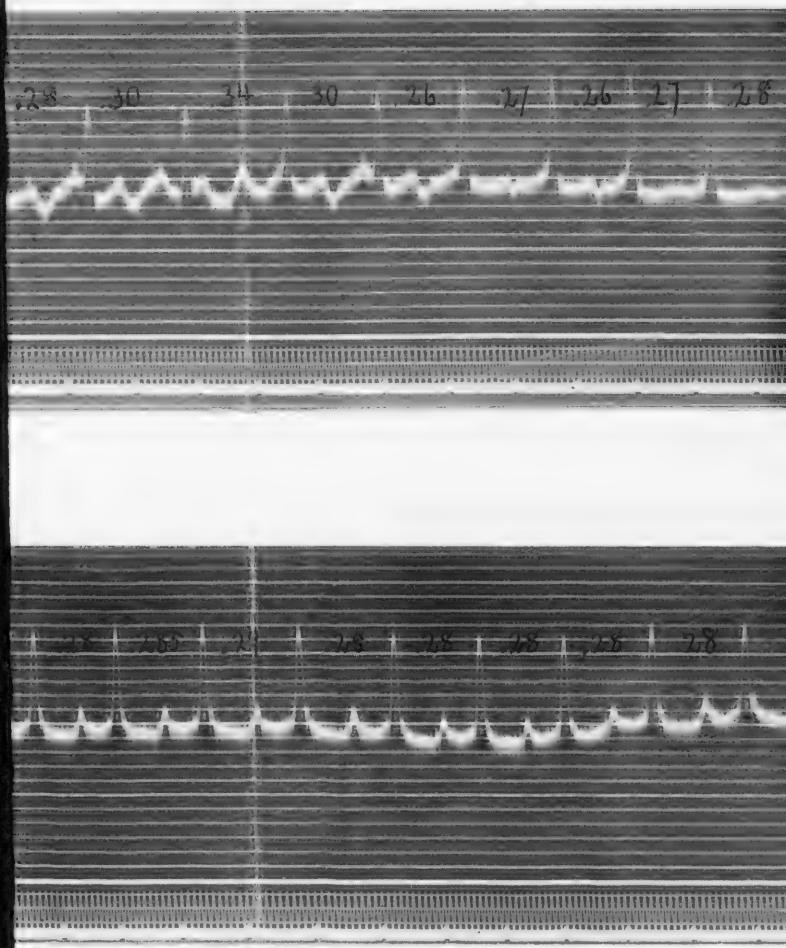
A



B

FIG. 10.

PLATE 6



(Robinson: Auricular Activ

THE EFFECT OF MORPHIN ON THE MECHANISM OF THE DOG'S HEART AFTER REMOVAL OF ONE VAGUS NERVE.*

By ALFRED E. COHN, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

PLATES 69 TO 73.

Van Egmond (1), Eyster and Meek (2), and Einthoven and Wieringa (3) have recently published observations on certain arhythmias which occurred in dogs after the intravenous injection of morphin. Einthoven and Wieringa showed that the alteration in the heart's action, due to the injection, depended on the fact that morphin stimulated the cardio-inhibitory center. But the resulting arhythmias were not uniform and they believed that the inconstant results arose because the drug sometimes selected one and sometimes another set of nerve fiber bundles for its predominant effect ("ungleichartige Vaguswirkung"). It seemed to the author that some of their curves resembled certain ones of his, obtained when the right vagus nerve was stimulated (4), and that others resembled those seen after stimulation of the left one, the other nerve in each case being cut. For that reason it occurred to him that morphin must have exerted its influence on the right or on the left cardio-inhibitory center in turn, or on both together; in the one case producing simple results easy to classify according to the center temporarily stimulated, or confused results when both centers were stimulated equally. It seemed natural to suppose if only one center, the right, for example, were stimulated, that the resulting arhythmia would have the same characteristics as that which was obtained on faradizing the right vagus nerve, when the left one was divided. In order to test this hypothesis, dogs, which had only one vagus nerve, were injected with morphin. In this way the stimulated center communicated with the heart by one nerve tract only.[1]

METHOD AND RESULTS.

In the present series of experiments, the plan consisted in excising one vagus nerve aseptically and then in testing the effect on the heart's mechanism of stimulating doses of morphin injected intra-

* Received for publication, July 12, 1913.

[1] Cohn, A. E., *Proc. Soc. Exper. Biol. and Med.*, 1913, x, 93.

venously. The similarity between the experimental conditions in the earlier and in the present series of experiments lies in the fact that in both one vagus nerve was stimulated (peripherally by a faradic current in the earlier, or centrally with morphin in the present series), and the other was divided. The study consisted, therefore, in a comparison of the curves resulting from morphin injections in the one vagus dog with those obtained by faradizing one vagus nerve.

Twenty dogs were studied in this series. The injections did not always result in that change in mechanism of the heart which has been described as characteristic of the result of injecting morphin (Nos. 683, 685, and 700). In these the rate was only slowed. Before an arhythmia characteristic of the group to which the dog belonged was observed, two (Nos. 686, 688, 699, 703, 705, and 715) or three (Nos. 706 and 721) experiments on different days were necessary. In seven experiments (697, 698, 702, 704, 718, 719, and 720) the mechanism was altered on the first injection. Part of the left vagus nerve was removed aseptically in ten, while in eight others a portion of the right nerve was excised. The dogs will be referred to according to the nerve which was left in place. The effect of freezing the nerves in two other dogs will be described. Except for the two last mentioned, all the dogs studied remained alive.

In nine experiments (Nos. 703, 704, 705, 706, 715, 718, 719, 720, and 721) morphin was injected, and characteristic morphin arhythmias occurred before excision of the vagus nerve. When the arhythmia had set in, operation for the removal of the right (Nos. 706, 715, and 721) or of the left vagus nerve (Nos. 703, 704, 705, 718, 719, and 720) was begun. In the dogs not enumerated here, the operations were performed before the injections were made. The dogs were anesthetized with ether and a skin incision was made. Eyster and Meek state that anesthesia suffices to abolish the arhythmia once it has been produced. We have seen several instances where this occurred, especially in a dog which stopped breathing and in which rather violent artificial respiration was practised. When he was restored, the arhythmia had disappeared. In other dogs morphin arhythmia disappeared only after

further interference, such as incision, manipulation of the tissues in the neck, or excision of a portion of one of the vagus nerves. In another dog (No. 715) a true arhythmia did not appear until the anesthesia was begun. It is probable, therefore, that anesthesia alone does not always inhibit the stimuli from the vagus centers, but that manipulation or operative procedures usually suffice to do so. The facts suggest the presence of an antagonism between the cardio-inhibitory stimulant (morphin) and the stimuli reaching the cardio-inhibitory center from the periphery. At the time of operation the latter outweigh and destroy the former. That the explanation offered is possible follows from observations made in six of the dogs (Nos. 702, 704, 705, 718, 719, and 720), in which the arhythmia produced by morphin disappeared after the beginning of the operation, but reappeared spontaneously, without the injection of any more morphin, at short intervals after the termination of the operations. In the four other dogs (Nos. 703, 706, 715, and 721) the arhythmia which was produced disappeared during the operation, but morphin injections on subsequent days resulted in arhythmias. The nature of the arhythmia, whether obtained directly after the operation or on an occasion subsequent to the operation, often differed from that obtained before the excision of one or the other of the vagus nerves. The difference in the arhythmia seen before operation and that observed afterward was especially striking in several dogs. Before operation, morphin injection caused a rhythm more or less characteristic of that seen in dogs having only one vagus nerve. After the excision of part of that nerve a rhythm typical of dogs retaining the opposite vagus nerve appeared.

The varieties of arhythmia which can be obtained by injecting morphin intravenously in dogs and before one of the vagus nerves has been removed have been described by Einthoven and Wieringa and by Eyster and Meek. Most of them have been seen in the course of the present series of experiments. After the excision of one vagus nerve, the confused and inconsistent results are not again seen. In all the experiments except three (Nos. 683, 685, and 700) which showed only sinus irregularity, arhythmias were obtained which could readily be classified as due to the influence exerted by one or the other vagus nerve. In one group the auricles continued

to beat at a moderately reduced rate and the ventricles followed them in sequence, but the interval between them (P–R time) was irregular, usually prolonged, the prolongation increasing as it does in digitalis poisoning. Occasionally and sometimes frequently, auricular contractions occurred which were not followed by contractions of the ventricles, and then a condition of partial auriculoventricular block (2:1 or 3:1) appeared (No. 720). The ventricular complexes resembled the normal controls. In two experiments (Nos. 702 and 703) the auricular rate was reduced and the conduction time much prolonged. At times it was so prolonged that the ventricles seemed not to receive the impulses resulting from auricular contractions, and therefore developed an independent rhythm. The arhythmia was one of temporary complete auriculoventricular dissociation, due to a defect in conduction rather than to the reduction in rate. Forms of arhythmia of the varieties described occurred in left vagus dogs only. They are therefore taken to be characteristic of left vagus dogs (table I).

In the second group the rate of the auricular contractions decreased distinctly. When the full effect of morphin was established the rate of auricular contractions decreased further. During this period efforts to establish an independent rhythm were constantly made by an ectopic ventricular focus. At the end of ten to twenty minutes, the auricular rate was exceedingly slow and an idioventricular rhythm was present. There was usually only a moderate or no defect in conduction, for when an auricular contraction occurred in that part of the cardiac cycle in which a ventricular response was possible, the ventricles responded after a normal interval and were represented by complexes shaped like those in the control curves. That the normal conduction time under these circumstances was not due to temporary freedom from fatigue is shown in curves where groups of escaped auricular contractions followed by ventricular beats did not show increasing conduction time in the successive cycles (Nos. 688 and 715). That no serious defect in conduction existed was also indicated by the fact that auricular contractions, the result of retrograde stimuli[2] from the independently

[2] A paper by Cohn and Fraser dealing with so called retrograde stimuli will appear in a forthcoming number of *Heart*.

beating ventricles, occurred (Nos. 688 and 698) after conduction intervals, the duration of which was within normal limits. This rhythm consists, therefore, in a slow auricular rate, independent ventricular contractions at a more rapid rate, and normal conduction time. This form of irregularity occurred in right vagus dogs only. It is taken to be characteristic of right vagus dogs.

When no change in the mechanism of the heart occurred (Nos. 683, 685, and 700), the rate was much reduced. The rhythm of the slow rate was not regular, but consisted in successions of cycles very long in duration followed by groups relatively much shorter. This rhythm has been described by Eyster and Meek. When they obtained it, they attempted to convert it into the cardiac arhythmia considered characteristic of morphin intoxication by Einthoven by injecting atropin subcutaneously. They found that between the time of injection and the establishment of the full atropin effect, there was a brief period during which an arhythmia was detected, and this rhythm they included among those produced by morphin. Atropin used in this way was employed in two instances (Nos. 683 and 685) in the present series, one a left and the other a right vagus dog. The resulting mechanism was identical in both. It consisted in incomplete auriculoventricular dissociation, similar to that shown in the curve in Eyster and Meek's paper. In view of the fact that differences were regularly found, with the exceptions noted, between right and left vagus dogs, when morphin alone caused an arhythmia, the method was abandoned. The use of atropin seemed ill advised also, because the site of action in the organism of the two drugs differs, morphin acting centrally and atropin peripherally. It seemed unlikely that the gradual paralysis of the vagus ends could yield fuller information regarding the results of stimulating the vagus center than had been obtained by injecting morphin alone. No attempt is made to explain the slow rhythm which Eyster and Meek and the present writer obtained. They have provided an explanation, based on the occurrence of sino-auricular blocks, which is not entirely satisfactory. This rhythm will be referred to as a sinus irregularity.

There were, as has been said, two experiments in which the vagi were not divided, but in which the impulses from the vagus centers

to the heart were blocked by freezing the nerves with ethyl chloride. The results of these experiments (Nos. 701 and 702) are given in detail here. The first is without interest because morphin was injected after the beginning of the operation and after both nerves were exposed, and because no effect was produced on the heart's rhythm. The second dog (No. 702) (figures 1 to 3) weighed 6.5 kilos. Morphin sulphate, twenty-four milligrams, was injected and a characteristic morphin arhythmia resulted resembling that due to stimulating the right vagus nerve (figure 2). Ether was given during the operation. Both vagus nerves were dissected and exposed and secured by strings placed about each, so that they could be elevated above the surrounding parts. Five minutes after the beginning of anesthesia, a curve was taken and demonstrated the continuance of a morphin arhythmia. But a curve taken directly after the conclusion of the dissection showed a normal rhythm. Three minutes later the morphin arhythmia returned. The right vagus nerve was elevated by its loop and a curve then made showed that the morphin rhythm was present. The right vagus nerve was then frozen and the morphin arhythmia disappeared entirely. After a lapse of three minutes, an arhythmia reappeared, but the rhythm had now changed from the one which had been present before freezing the right vagus nerve to one characteristic of stimulating the left vagus nerve (figure 3). Three curves were made showing this rhythm and, seventeen minutes later, the left vagus nerve was elevated, as the right vagus had been, above the surrounding tissues. A curve now made showed that the left vagus rhythm was still present. The left vagus nerve was then frozen. The morphin effect immediately disappeared. It did not return, for curves were made at intervals for the next six hours and again on the following day. It will be seen that morphin produced a disordered rhythm in this dog which was not destroyed by anesthesia, but which disappeared later in the operation; that the disordered rhythm reappeared soon after the end of the operation; that freezing the right vagus produced an arhythmia characteristic of those seen in left vagus dogs; and that freezing the left nerve destroyed this arhythmia entirely. Freezing, therefore, produced an effect like section of the nerve, and subsequent thawing was not, as cooling usually is,

followed by a return of function in the nerve. The non-return to function of the nerves as a result of freezing was not anticipated, but its importance lies in the opportunity which the method offers for the study of selected fibers not destroyed by this injury. This experiment may then be added to those showing a morphin arhythmia typical of left vagus dogs.

PROTOCOLS.

Experiment 683.—Left vagus dog; female.

Oct. 24, 1912. 1.5 cm. of the right vagus nerve were excised.

Jan. 10, 1913. Weight 9.5 kilos. The rate of the heart was 191.3 per minute, the conduction time was 0.1 of a second. There was a slight sinus arhythmia. Morphin sulphate, 64 mg., was injected into an ear vein. In 1 minute the rate was 165 and the conduction time 0.18 of a second. In 16 minutes the rate was 161.1 and the conduction time was 0.1 second. The sinus arhythmia was slight. In 90 minutes no change in rate or rhythm had taken place, so morphin, 30 mg., was again injected. In 126 minutes (36 minutes after the second injection) the rate was 106.3 and the conduction time 0.12 of a second. The sequence of auricular and ventricular systoles remained normal, but a marked arhythmia took place. This consisted in a group of rapid, followed by a group of slower cycles. It is this rhythm which is described as a sinus irregularity. Morphin sulphate, 72 mg., was injected a third time 132 minutes after the first injection. The rate at first rose to 200, the conduction time being 0.11 second, but fell gradually until 7 hours after the first injection the rate was 66.3 and the conduction time 0.11 to 0.12 of a second. The next morning the rate was 127.2 and the conduction time 0.11 of a second.

Feb. 13, 1913. Second experiment. Morphin sulphate, 24 mg., was injected and produced an arhythmia similar to that of the former experiment. Atropin sulphate, 1.2 mg., was then injected subcutaneously. Increase in rate gradually took place. In 12 minutes incomplete dissociation occurred at a ratio of 2:1, but was seen for 2 or 3 minutes only. This rhythm was associated with lengthened conduction time, which persisted a short time (2 or 3 minutes). In 18 minutes after atropin was injected, the heart was released from inhibition.

Summary.—In this dog morphin produced a slow sinus irregularity, except that just after the first injection the conduction time was much increased. During the release by atropin from increased inhibition due to morphin, a 2:1 rhythm and lengthened conduction time were observed.

Experiment 685.—Right vagus dog; female.

Oct. 31, 1912. 1 cm. of the left vagus nerve was excised by Dr. Robinson.

Jan. 20, 1913. Morphin sulphate, 24 mg., was injected into an ear vein, but aside from the occurrence of a slow sinus irregularity (the rate fell from 122.7 to 85.7 beats), no abnormality was recorded.

Jan. 27. Second experiment. The same quantity of morphin was injected, with the same results as on the first occasion.

Feb. 13. Third experiment. An injection of morphin, 24 mg., reduced the

rate to 72.8 and produced a rhythm like that already noted. Atropin sulphate, 1.2 mg., was then injected subcutaneously and in 10 minutes the rate increased to 160 and only every second or third auricular contraction was followed by a ventricular beat. There was incomplete 2:1 and 3:1 auriculoventricular block (figure 4). 13 minutes after the injection of atropin, the rate was 240.16, the rhythm was normal, and the conduction time was 0.12 of a second.

Summary.—This dog did not differ from No. 683 in the rhythm produced by morphin or in the nature of the release resulting from the injection of atropin. A difference exists, however, in the fact that in No. 683, a left vagus dog, the conduction time rose after the injection of morphin, but did not change in this one.

Experiment 686.—Right vagus dog; female.

Oct. 31, 1912. 1 cm. of the left vagus nerve was excised by Dr. Robinson.

Jan. 21, 1913. Weight 6 kilos. The heart rate was 266.1 and the conduction time 0.09 of a second. Morphin sulphate, 24 mg., was injected intravenously. In 95 minutes the rate, after falling gradually, was 60.4 and the conduction time varied from 0.09 to 0.11 of a second. There was a sinus irregularity.

Feb. 13. Second experiment. The rate was 161 and the conduction time 0.1 of a second. Morphin sulphate, 24 mg., was injected and in 20 minutes the rate had fallen to 50.1, the conduction time remaining unchanged. At this time ectopic contractions, arising in the wall of the left ventricle, began to appear. They occurred after a delay and were not premature as are extrasystoles (premature beats). They forestalled the delayed auricular contractions when these occurred after an unusually prolonged interval. The auricular rate became constantly slower, and the auricular contractions occurred as single beats or in groups of two or three at long intervals. In 35 minutes the rate of the auricular beats was 32.3 (figure 5). When they were followed by normal ventricular systoles, the conduction time was normal. The ventricular rate, counting both normal and ectopic beats, was 48.1. Morphin, 24 mg., was now injected, and the arhythmia disappeared promptly.

Summary.—The first morphin injection resulted merely in a slow sinus irregularity. But the second injection caused a slowing of auricular contractions so great that the opportunity for an independent ventricular rhythm was afforded. The injection of more morphin destroyed the arhythmia which had been produced. Einthoven and Wieringa have described this phenomenon. The arhythmia which was produced is held to be characteristic for that produced by morphin in right vagus dogs.

Experiment 688.—Right vagus dog; female.

Nov. 7, 1912. 1 cm. of the left vagus nerve was excised by Dr. Robinson.

Jan. 9, 1913. Weight 8.4 kilos. The heart rate was 168.7 and the conduction time 0.09 of a second. Morphin sulphate, 48 mg., was injected intravenously. There was a temporary increase in rate, but none in conduction time. In 30 minutes the rate was 97.5 and the conduction time 0.1 of a second. In 56 minutes the rate was 72.4 and the conduction time 0.1 to 0.11 of a second. In 59 minutes the rate was 62 and the conduction time 0.11 to 0.13 of a second. A few ectopic contractions arose in the wall of the left ventricle. These were not premature, but occurred during a long auricular pause. There was marked sinus irregularity. In 70 minutes the auricular rate had fallen to 49 and many contractions arising in the wall of the left ventricle were seen (figure 6). The

complexes which represent these show varying relations to the auricular (P) waves. In 144 minutes the auricular rate, in so far as it could be determined, was between 20 and 40 a minute, while the ventricular rate was 72. The calculation of the ventricular rate included both the usual complexes and also the atypical left ventricular ones. The rhythm was made of successions of two or three cycles followed usually by three atypical ones. The conduction time in the normal ones was 0.11 to 0.12 of a second; the atypical beats were dissociated from the auricular contractions.

Feb. 14, 1913. Weight 7.2 kilos. The rate was 127.2 and the conduction time 0.11 to 0.12 of a second (figure 7). An injection of morphin, 32 mg., was given in an ear vein. The conduction time was not increased. In 6 minutes the rate had fallen to 68.4, but the conduction time remained 0.11 of a second. Independent atypical (left ventricular) contractions, like those in the former experiment, began to appear. These, as were almost all of the independent left ventricular contractions in this case, were followed by auricular contractions after an interval of 0.14 to 0.16 of a second. The complex representing these auricular beats was inverted. They were due to retrograde stimuli from the ventricles. In 22 minutes the auricular rate had fallen to 11 a minute (figures 8 and 9). The circulation was maintained by independent left ventricular contractions at a rate of 55.7. In 35 minutes the auricular rate was 6 and the ventricular rate 53.3. This rhythm remained unchanged for 83 minutes after the injection, when the ventricular rate was 52.8 and no auricular contractions could be demonstrated.

Discussion.—The calculation of the auricular rate given in this experiment is made on several grounds. A comparison of the undeformed left ventricular complexes with those that are deformed leads to the conviction that the negative waves inserted between R and T are P waves, and follow the R waves at definite intervals. There can be little doubt that they are stimulated by the ventricular contractions which immediately precede them. Being dependent on the ventricles, they are not included in estimating the independent auricular rate. The other P waves in these curves are positive and when they occur (figure 8) are usually followed by ventricular complexes of normal outline after a normal interval (0.13 of a second). The P waves which are followed by normal ventricular complexes occur within a definite period of ventricular diastole. When they fall outside this period, they do not influence the rhythm of the independently beating ventricles, and are readily identified (figure 8).

Summary.—In this dog morphin caused a great reduction in the auricular rate. It was so slow that from the viewpoint of the mechanism of the heart, the auricles may be regarded to have ceased to beat. The ventricles developed an independent rhythm. The auriculoventricular conduction time remained normal. There were retrograde auricular systoles. The arhythmia was characteristic of those produced when the right vagus nerve is stimulated.

Experiment 697.—Right vagus dog; male.

Jan. 6, 1913. 1 cm. of the left vagus nerve was excised.

Feb. 14, 1913. Weight 7.5 kilos. The heart rate was 135.5 and the conduction time 0.1 to 0.11 of a second. Morphin sulphate, 32 mg., was injected into an ear vein. The rate did not rise, nor did the conduction time change. In 34 minutes the rate of the auricular contractions fell to 53.3 a minute. There was great

irregularity of the rhythm of the auricles. Many of the auricular beats, especially those occurring at periods of greater frequency, were followed by normal ventricular contractions. But independent contractions arose in the walls of the left ventricles, either singly or in pairs. Their rate was 58.86. In 48 minutes a second injection of morphin, 16 mg., was given to slow the auricular rate more. In 76 minutes (28 minutes after the second injection), this was reduced to 41.52 and the ventricular rate was 57.3. The nature of the rhythm was unchanged and the conduction time remained 0.09 to 0.1 of a second.

Summary.—Morphin decreased the auricular rate. Independent ventricular beats occurred. The rate of the ventricular beats was faster than the slowed auricular rate. The nature of the rhythm was similar to that in dog 688. The arhythmia was characteristic of those produced when the right vagus nerve is stimulated.

Experiment 698.—Right vagus dog; male.

Jan. 6, 1913. 1 cm. of the left vagus nerve was excised.

Jan. 24, 1913. Weight 6.6 kilos. The heart rate was 95.9 and the conduction time 0.12 of a second (figure 10). Morphin sulphate, 24 mg., was injected intravenously. In 1½ minutes the auricular rate was 30, the conduction time remained 0.12. Many contractions arose in the wall of the left ventricle, beating in complete dissociation from the auricles. A few ventricular beats were followed by retrograde auricular contractions. The nature of the undeformed ventricular complexes (figure 11) was established by studying the relations of the independent P waves to these. In 5 minutes the auricular rate was 12 and the independent ventricular rate was 65. There were no sequential ventricular beats. The auricular beats were all independent, and no auricular beats due to retrograde stimuli from the ventricles were identified. For 39 minutes no auricular contractions (figure 12) were demonstrable in the seven curves made at about this time. The ventricular rate varied from 64.6 to 61.8. In 44 minutes independent auricular waves were again observed (figure 13), but the P waves were now negative. The relation of these to the ventricular complexes also serves to show that the latter are not deformed by auricular or other unidentified deflections. In 57 minutes the auricular rate was 50 and the ventricular 60.09. Recovery with the reappearance of normal cycles then occurred, the conduction time at first being 0.18 of a second.

Summary.—Morphin injection caused slowing, possibly stoppage, of the auricles; and an independent ventricular rhythm at the rate of 60 to 65 appeared. On recovery, negative P waves were observed. The conduction time was lengthened at first. The arhythmia was characteristic of those produced when the right vagus nerve is stimulated.

Experiment 699.—Left vagus dog; male.

Jan. 7, 1913. 1 cm. of the right vagus nerve was excised.

Jan. 14, 1913. Weight 8.5 kilos. The heart rate was 151.4 and the conduction time was 0.1 of a second. Morphin sulphate, 32 mg., was injected and the rate rose in 2 minutes to 192.3, and the conduction time fell to 0.09 of a second. In 7 minutes the rate fell to 60, the conduction time rose to between 0.14 and 0.16 of a second, and an occasional auricular contraction was blocked, resulting in a 2:1 rhythm. A record of one such blocked portion was obtained. Sinus irregularity was present. Long conduction time, 0.12 to 0.16, was observed for

about 35 minutes. In 42 minutes the rate was rising, 81.2, and the conduction time falling. A second injection of 48 mg. and a third one of 80 mg. produced no increase in the degree of arhythmia.

Jan. 27, 1913. Second experiment. The rate was 156.8 and the conduction time 0.09 to 0.1 of a second. Morphin sulphate, 24 mg., was injected. In 1 minute the rate was 188.4 and the conduction time 0.09 of a second. In 13 minutes the rate was 62.3 and the conduction time rose, and varied between 0.13 and 0.16 of a second. An auricular contraction was blocked. The lowest rate noted occurred in 19 minutes and was 55.8; the conduction time was 0.13 to 0.18 of a second. There was blocking of auricular contractions. A progressive increase in the conduction time, followed by a blocked auricular wave, often seen in digitalis intoxication, was not seen. It happened occasionally that a blocked auricular systole was followed by a long delayed auricular beat. A premature ventricular beat, arising in the right ventricle, forestalled this contraction. A second injection of morphin, 24 mg., caused no increase in the degree of arhythmia.

Summary.—Morphin slowed the heart rate, increased the conduction time, and produced incomplete heart block in each of two experiments. Increasing the quantity of morphin did not increase the degree of effect. The arhythmia was characteristic of those produced when the left vagus is stimulated.

Experiment 700.—Left vagus dog; male.

Jan. 7, 1913. 1 cm. of the right vagus nerve was excised.

Jan. 16. First experiment. The dog weighed 6.4 kilos. The heart rate was 95.7 and the conduction time was 0.11 to 0.12 of a second. Morphin sulphate, 19.2 mg., was injected and in 1 minute the rate rose to 206.1 and the conduction time fell to 0.07 of a second. In 18 minutes the rate fell to 91.4 and the conduction time was 0.1 of a second. In 59 minutes the rate was 97.2 and the conduction time 0.11. Morphin sulphate, 20 mg., was injected again. The rate rose to 204.5 and the conduction time fell to 0.07 of a second. Only a sinus irregularity was produced.

Jan. 28. Second experiment. The weight of the dog was 6.2 kilos. The heart rate was 208.9 and the conduction time 0.08 to 0.09 of a second. Morphin sulphate, 24 mg., was injected. Directly afterwards, the rate rose to 278.5 and the conduction time fell to 0.07 of a second. In 20 minutes the rate was 196 and the conduction time 0.07 to 0.08. A second injection of morphin, 160 mg., was given and the rate rose to 215 and the conduction time to 0.1. In 83 minutes the rate had risen further to 231.2, while the conduction time had fallen to 0.07 of a second. An intravenous injection of atropin sulphate, 1.8 mg., raised the rate to 260. The conduction time remained unchanged. In 169 minutes (95 minutes after the injection of atropin) the rate was 200 and the conduction time 0.08.

Feb. 17. Third experiment. The rate was 247.2 and the conduction time was 0.07 to 0.09 of a second. Morphin sulphate, 40 mg., was injected. In 1 minute the rate rose to 253 and the conduction time fell to 0.06 of a second. In 98 minutes the rate fell to 93.5 and the conduction time was again 0.07 to 0.08.

Summary.—Morphin produced a slowing of the heart and a sinus irregularity. Three attempts failed to produce a change in the relation of auricular and ventricular contractions.

Experiment 703.—Left vagus dog; female.

Jan. 30, 1913. First experiment. Weight 6.8 kilos. The rate of the heart was 104.2 and the conduction time 0.09 to 0.1 of a second. Under anesthesia the rate fell to 85.8. A part (2 cm.) of the right vagus nerve was excised. After excision, the rate was 151.9 and the conduction time 0.08 of a second. After the anesthesia the rate was 202.6 and the conduction time 0.08 of a second. Morphin sulphate, 16 mg., was injected. In 25 minutes the rate was 69.3 and the conduction time 0.13 to 0.14 of a second. In 29 minutes, morphin sulphate, 16 mg., was again injected. The rate rose at first to 91.6 and the conduction time fell to 0.1 to 0.11, but in 45 minutes (16 after the second injection) the rate had again fallen to 53.1, the conduction time remaining unchanged. A third injection of morphin sulphate, 16 mg., was injected 49 minutes after the first one, but the rate and conduction remained unchanged (49.2 and 0.1 to 0.11 of a second). Atropin sulphate was then given intravenously and the rate rose within a minute to 193.3 and the conduction time fell to 0.09.

Feb. 17. Second experiment. The rate of the heart was 145.8 and the conduction time 0.09 of a second. Morphin sulphate, 40 mg., was injected. In 2 minutes the rate fell to 89 and the conduction time was 0.1 to 0.11. In 2½ minutes there were 40 auricular and 50 ventricular systoles. Most of the ventricular contractions arose in the wall of the right ventricle and were independent of the auricular beats. Some of the ventricular beats, however, resulted from stimuli arising in the auricles. The conduction time in such cycles was 0.16 to 0.18 of a second. In 25 minutes the auricular rate was 37.12 and the ventricular rate was 52.3. A few auricular contractions were followed by ventricular beats at intervals varying from 0.16 to 0.20 of a second. Except for these, there was As–Vs dissociation. In 39 minutes the auricular rate was 28.5 and the ventricular 48.4. Groups of normal cycles appeared, the conduction time in which was 0.12 to 0.13 of a second. After this (in 139 minutes) the rhythm returned to normal. The auricular rhythm throughout was very irregular.

Summary.—In the first experiment the rate fell during anesthesia. After excision of the right vagus nerve and the cessation of the anesthetic the rate rose. Morphin slowed the rate and increased the conduction time. Subsequent injections of morphin produced very little further slowing in rate and left the conduction time normal. Atropin raised the rate promptly and reduced the conduction time very slightly. In the second experiment morphin slowed the auricular rate. The circulation was maintained in part by independent beats of the ventricles, the impulse to contraction arising in the right side of the heart. When an auricular contraction gave rise to one in the ventricles, the interval between the two was increased. The degree of block over the conduction system obviously varied; at times the propagation of impulses was merely delayed, at other times it was entirely stopped, so that auricular contractions, even when they fell in suitable portions of diastole, failed to elicit ventricular responses.

Discussion.—In no other left vagus dog was so low an auricular rate reached (table I). In such cases differentiation from right vagus dogs would be impossible, were it not for the marked increase in conduction time found in this dog. In the one right vagus dog in which the conduction time was as high, the auricular rate fell to 4 and so prevented a confusion.

Experiment 704.—Left vagus dog; female.

Jan. 31, 1913. Weight 9.2 kilos. The rate was 93.6 and the conduction time 0.1 of a second. Morphin sulphate, 24 mg., was injected and in 3 minutes a rhythm characteristic of morphin poisoning was established. In 15 minutes the auricular rate was 28.9 and the ventricular rate was 60. There were a number of cycles in which auricular beats were followed by ventricular ones. The conduction time in them varied between 0.12 and 0.14 of a second. Ether was administered, but the arhythmia continued. The rate of the auricular beats was 51.8 and of the ventricular 77.6. The normal cycles showed a conduction time of 0.17 to 0.19 of a second. An incision in the skin was made and directly afterward the normal rhythm was reëstablished. The curve now taken, 21 minutes after the injection of morphin, showed a rate of 110.1 and a conduction time of 0.13 of a second. A part (2 cm.) of the right vagus nerve was excised. The anesthesia and the operation ended. In 32 minutes (1 minute after the end of the operation) the heart rate was 150 and the conduction time 0.1 to 0.11 of a second; the rhythm was normal. In 63 minutes (32 minutes after the operation) an arhythmia appeared, but its nature differed from that seen before operation. It consisted in incomplete heart-block at a ratio of 2:1. The blocks occurred after a succession of cycles in which the coördination was normal, but the conduction was prolonged from 0.12 to 0.18. The conduction time in the cycle before the blocked auricular contraction showed the longest interval. This rhythm continued for 123 minutes after the end of the operation. The rate was 95.5 and the conduction time 0.11 to 0.18 of a second. Morphin sulphate, 24 mg., was injected again. The rate rose to 114.2 and the conduction time was 0.12 to 0.14 of a second. There were no blocked auricular beats, but these reappeared a minute later, the rate falling to 90. A third injection of morphin, 24 mg., was given 197 minutes after the first (166 minutes after the end of the operation), but produced no change in the rhythm. In 18 minutes morphin, 128 mg., was again injected and this time destroyed the arhythmia permanently. The rate was 124.3 and the conduction time 0.1 to 0.11 of a second, falling in 28 minutes to 107.3, the conduction time being 0.11 of a second (table II).

Summary.—Morphin caused a disordered rhythm which was destroyed after the skin incision for the excision of the right vagus nerve was made. A part of the right vagus nerve was excised and the operation ended. In half an hour after the operation, incomplete heart-block (2:1) developed and lengthened conduction time appeared. The rate of the auricles was the same as before morphin injection. Subsequent injections did not change the nature of the arhythmia. A final large dose destroyed it. The arhythmia was characteristic of that produced when the left vagus nerve is stimulated.

Experiment 705.—Left vagus dog; male. Weight 6.6 kilos.

Feb. 3, 1913. Morphin sulphate, 24 mg., was injected, but resulted only in sinus irregularity. The experiment was discontinued.

Feb. 7. Second experiment. The rate was 105.8 and the conduction time 0.1 to 0.11 of a second. Morphin sulphate, 24 mg., was injected. In 7 minutes the rate was 57.8 and the conduction time 0.11 to 0.13 of a second. A few premature contractions arose in the wall of the right ventricle. In 18 minutes, morphin sulphate, 24 mg., was again injected. In 20 minutes (2 minutes after the second injection) the rate was 46 and the conduction time 0.12 of a second. There were now many premature contractions arising in the same site as before.

TABLE I.

No. of animal.	Injection before operation.	Date of operation.	Date of first injection.	Date of second injection.	Date of third injection.	Before injection.		After injection.						Rhythm.
						Rate.	Conduction.	Highest rate.			Lowest rate.			
								As.	Vs.	P-R.	As.	Vs.	P-R.	
685	−	Oct. 31, 1912	Jan. 20, 1913	Jan. 27, 1913	Feb. 13, 1913	122.7	0.11	258.3	258.3	0.1	85.7	85.7	0.1	Sinus irregularity.
686	−	Oct. 31, 1912	Jan. 21, 1913	Feb. 13, 1913		161.0	0.1	206.8	206.8	0.1	32.3	48.1	0.11–0.12	Right vagus.
688	−	Sept. 7, 1912	Jan. 9, 1913	Feb. 14, 1913		127.2	0.11–0.12	148.8	148.8	0.1	6–11	53.3	0.12–0.13	Right vagus.
697	−	Jan. 6, 1913	Feb. 14, 1913			135.5	0.1–0.11	133.5	133.5	0.1–0.11	41.52	57.3	0.09–0.11	Right vagus.
698	−	Jan. 6, 1913	Jan. 24, 1913			95.9	0.12	40.0	65.0	0.13–0.15	0–12	61.8		Right vagus.
706	+	Feb. 3, 1913	Feb. 3, 1913	Feb. 4, 1913	Feb. 6, 1913	134.1	0.1	78.4	97.0	0.09–0.14	10.0	75.3	0.11	Right vagus.
715	+	May 23, 1913	May 23, 1913	June 5, 1913		126.5	0.14	158.8	158.8	0.09–0.11	4–30	61.9	0.12–0.18	Right vagus.
721	+	June 13, 1913	June 11, 1913	June 13, 1913	May 23, 1913	230.0	0.09	163.3	163.3	0.09	36.3	46.4	0.11–0.12	Right vagus.
683	−	Oct. 24, 1912	Jan. 10, 1913	Feb. 13, 1913		191.3	0.1	165.0	165.0	0.18	66.3	66.3	0.11–0.12	Sinus irregularity.
699	−	Jan. 7, 1913	Jan. 14, 1913	Jan. 27, 1913		156.8	0.09–0.1	188.4	188.4	0.09	56.5	2:1	0.11–0.18	Left vagus.
700	−	Jan. 7, 1913	Jan. 16, 1913	Jan. 28, 1913	Feb. 17, 1913	95.7	0.11–0.14	206.1	206.1	0.07–0.08	91.4	91.4	0.1	Sinus irregularity.
702	+	Jan. 24, 1913	Jan. 24, 1913			134.0	0.12	213.0	213.0	0.09	72.6	77.4	0.16–0.17	Complete heart-block.
703	−	Jan. 30, 1913	Jan. 30, 1913	Feb. 17, 1913		145.8	0.09	89.0	89.0	0.1–0.11	28.5	48.0+	0.16–0.2	Complete heart-block.
704	+	Jan. 31, 1913	Jan. 31, 1913			93.6	0.1	150.0	150.0	0.1–0.11	91.3	2:1	0.12–0.18	Left vagus.
705	+	Feb. 7, 1913	Feb. 3, 1913	Feb. 7, 1913		105.8	0.1–0.11	176.6	176.6	0.14	80.0	2:1	0.12–0.18	Left vagus.
718	+	May 29, 1913	May 29, 1913			107.5	0.11	173.7	173.7	0.1	95.2	2:1	0.12–0.16	Left vagus.
719	+	June 9, 1913	June 9, 1913			100.0	0.12–0.14	245.6	245.6	0.08–0.09	107.1	2:1	0.12–0.18	Left vagus.
720	+	June 10, 1913	June 10, 1913			85.7	0.12–0.14	200.8	200.8	0.1	117.7	2:1–3:1	0.11–0.18	Left vagus.

The first group, Nos. 685 to 721, are right vagus dogs.
The second group, Nos. 683 to 720, are left vagus dogs.

Ether was administered. All the ventricular contractions, except two, now arose in the wall of the right ventricle. Their rate was 91, while that of the auricular contractions was 41.8. The two normal ventricular beats mentioned followed auricular beats at intervals of 0.16 and 0.15 of a second. Artificial respiration became necessary and was used for a few minutes. After recovery (32 minutes after the first morphin injection) the rate was 80.8 and the conduction time 0.13 to 0.14. No abnormal beats were seen. The right vagus nerve, 2 to 3 cm., was then removed and the operation ended (56 minutes after the first injection). Directly after the operation (59 minutes after the first injection) the rhythm was still normal. The rate was 176.6, the conduction time 0.14 of a second. In 81 minutes (25 minutes after the end of the operation) the rate was 136.8 and the conduction time 0.12 to 0.16 of a second. One blocked auricular contraction was seen. In 107 minutes (51 minutes after the end of the operation) the rate was 113.1; the conduction time varied between 0.12 and 0.16. There were now many blocked auricular beats, the rhythm being 2:1 and occasionally 3:1. In 116 minutes (60 minutes after the end of the operation) morphin sulphate, 24 mg., was again injected. The rate continued to fall and in 190 minutes (134 minutes after the end of the operation) the rate was 78.5 and the conduction time 0.14 to 0.16 of a second; there were many blocked auricular beats forming a 2:1 rhythm.

Summary.—Morphin caused characteristic arhythmia resembling the effect of stimulating the right more than left vagus nerve, except for the fact that conduction time, where it could be studied, was lengthened. After artificial respiration the arhythmia disappeared; the conduction time was still slightly lengthened. After the operation incomplete heart-block continued to be observed for 134 minutes after its onset. The arhythmia was characteristic of those observed when the left vagus nerve was stimulated.

Experiment 706.—Right vagus dog; male.

Feb. 3, 1913. Weight 6.7 kilos. The rate was 86.3 and the conduction time was 0.08 to 0.09 of a second. An injection of morphin sulphate, 24 mg., raised the rate to 210 and lengthened the conduction time to 0.1 to 0.11 of a second. In 5 minutes the rate fell to 99.3; the conduction time remained unchanged. In 13 minutes morphin, 24 mg., was again injected. The rate continued to fall, In 34 minutes the rate was 61.9. There were numerous ventricular contractions, some having their origin in the wall of the right and others in the wall of the left ventricle. The rate of these ventricular beats was the same as that of the auricles, but a relation between the two cannot be determined; they were beating in complete dissociation. Ether was then administered and in 38 minutes the auricular rate had fallen further to between 20 and 40, while the ventricular rate rose to 103.4. All the ventricular beats arose now in the wall of the left ventricle and were completely dissociated from the auricles. Two auricular beats were followed by normal ventricular beats at intervals of 0.14 and 0.16 of a second. After the skin incision (48 minutes after injection) the rate of the auricular beats rose to 57.1 and that of the ventricular beats was 93.7. The latter arose in the wall of the left ventricle, except one which was of normal outline and followed the preceding auricular contraction in 0.18 of a second. The left vagus nerve was now dissected out (52 minutes after injection). The rate of the auricular systoles was then 73.5 and that of the ventricles 107.4. As before,

these arose in the wall of the left ventricle, except six cycles which were normal and had a conduction time of 0.1 to 0.12 of a second. Part of the left vagus (2 cm.) nerve was now excised and the operation ended. The rate (58 minutes after injection) of the heart was now 140 and the conduction time 0.06 to 0.08 of a second. There was no arhythmia. In 134 minutes the rate was 126.9 and the conduction time 0.06 of a second. The rhythm remained normal.

Feb. 4, 1913. Second experiment. The rate was 194.1 and the conduction time 0.08 to 0.09 of a second. During the experiment, morphin sulphate, 96 mg., was injected. Slow sinus irregularity was produced, but no arhythmia occurred.

Feb. 6. Third experiment. The rate of the heart was 134.1 and the conduction time was 0.1 of a second. Morphin sulphate, 24 mg., was injected and in 5 minutes the rate of the auricular beats fell to approximately 50. Their rhythm was irregular, consisting of groups of rapid beats associated with normal ventricular beats, the conduction time being 0.1 to 0.12 of a second, and others at a slower rate. During the groups of slow beats, many premature ventricular contractions, arising in the wall of the left ventricle, occurred. The rate of the ventricular beats was 91.1. In 35 minutes the rate of the auricular beats was 38.8 and that of the (left) ventricular beats 81.8, the latter dissociated from the auricular beats. There were 9 normal cycles in a rather long curve, the conduction time in these being 0.14 to 0.16. Morphin sulphate, 16 mg., was again injected. In 87 minutes the rate of the auricles fell to about 10 and the ventricular beats to 75.3. All the latter arose in the wall of the left ventricle. One normal cycle was seen at this time, the conduction time being 0.11 of a second. The number of normal cycles then increased and in 158 minutes a normal rhythm was again restored.

Summary.—Before operation, morphin caused an arhythmia of a confused type. Neither anesthesia nor the trauma resulting from operation destroyed the arhythmia. After excision of the nerve the arhythmia disappeared. The arhythmia did not return on this day. The next day an attempt to produce the arhythmia failed. Two days later morphin slowed the rate of the auricles and an independent ventricular rhythm arose. When an auricular contraction was followed by a ventricular contraction, the conduction time was normal. The arhythmia was characteristic of those produced when the right vagus nerve is stimulated.

Experiment 715.—Right vagus dog; male.

May 23, 1913. Weight 9.2 kilos. The heart rate was 126.5 and the conduction time 0.14 of a second (figure 14). An injection in an ear vein of morphin sulphate, 80 mg., in 2 minutes raised the auricular rate to 173.7 and reduced the conduction time to 0.11 of a second. After 17 minutes and again after 59 minutes, morphin sulphate, 72 mg. and 96 mg. (total, 248 mg.), was injected and no arhythmia was observed. The rate had fallen to 59 and the conduction time was 0.12 of a second. Ether was administered 75 minutes after the first injection. An arhythmia now appeared; it consisted of an auriculoventricular dissociation, the auricular rate being about 70 and the ventricular rate 92.6. The ventricular contractions took their origin in the wall of the left ventricle (figure 15). This rhythm continued a few minutes, but disappeared 9 minutes after it began (figure 16). The left carotid sheath had meanwhile been dissected out. The rate of the coördinated heart was 136.6 and the conduction time 0.12 of a second. 4 or 5 cm. of the left vagus nerve were excised. The heart rate was 171.1 and

the conduction time 0.11 of a second. 67 minutes later (161 minutes after the first injection) the rate of the heart was 98.8 and the conduction time 0.12 of a second. The experiment was discontinued.

June 5. Second experiment. The heart rate was 132.2 and the conduction time 0.12 of a second. Morphin sulphate, 40 mg., was injected into an ear vein. In 1 minute the rate rose to 158.8 and the conduction time was 0.1 of a second. In 3 minutes the rate was 107.08 and the conduction time 0.09 to 0.11 of a second. In 5 minutes the auricular rate had fallen to 46 and the ventricular rate was 55.7. The rhythm of the auricles was irregular and the long delays were anticipated a number of times by premature ventricular contractions arising in the wall of the left ventricle. In the coördinated cycles the conduction time was 0.12 to 0.14 of a second. In 17 minutes the rate of the auricular beats had fallen to about 30, while the ventricular rate was 61.4. These now arose in the wall of the right ventricle. Two sequential cycles which occurred at this time showed a conduction time of 0.19 of a second. Observation of the galvanometer string at the time of operation failed to detect the nature of the arhythmia. A second injection of morphin sulphate, 32 mg., was therefore given 21 minutes after the first. It destroyed the arhythmia; the heart rate was 108.4 and the conduction time 0.12 of a second. In 2 minutes the arhythmia reappeared. The rate of the auricular beats was about 30 and that of the ventricular 67.1 (figure 17). The origin of the ventricular beats was in the wall of the right ventricle. Four sequential cycles which appeared at this time showed a conduction time of 0.16 to 0.18 of a second. One minute later (25 minutes after the first injection) five auricular beats occurred in 3 seconds, but after that no auricular systoles took place for 15 seconds (figure 18). The rate of the ventricular beats was 61.9. Three normal cycles showed a conduction time of 0.14 to 0.16 of a second.

Summary.—When the dog was intact, morphin produced a rhythm characteristic of those seen when the left vagus nerve was stimulated. The arhythmia appeared only after the beginning of ether anesthesia. The production of the arhythmia, although two drugs were administered, was probably due to morphin. That it occurred after ether was given is probably accidental. The operative procedure destroyed the arhythmia. In a later experiment the arhythmia produced was characteristic of those observed when the right vagus nerve is stimulated, except for the prolonged conduction time mentioned.

Experiment 718.—Left vagus dog; female.

May 29, 1913. Weight 7.5 kilos. The heart rate was 107.5 and the conduction time 0.11 of a second. Morphin sulphate, 48 mg., was injected in an ear vein. In 2 minutes the rate was 173.75 and the conduction time 0.1 of a second. In 13 minutes the rate of the heart was 40 and the conduction time 0.12 of a second. A minute later, the rate being 43.7, during the long intervals between the irregular auricular systoles, two left ventricular beats occurred. In 25 minutes the rate of the auricular beats was 60 and that of the ventricular beats 71.03. There was pronounced sinus irregularity; in the long intervals between auricular beats, groups of two to four left ventricular beats occurred. The conduction time in the sequential cycles was 0.13 of a second. 12 minutes later (37 minutes after the injection) the rhythm was regular and normal, the rate 39.6 and the conduction time 0.13 of a second. During ether anesthesia, about 5 cm. of the right vagus nerve was excised. When the operation ended, 57 minutes after the

injection, the rate of the heart was 123.3 and the conduction time 0.12 to 0.13 of a second. 10 minutes later (67 minutes after the injection) the rate was 78.8 and the conduction time varied between 0.12 and 0.18. There were now blocked auricular beats, forming an incomplete heart-block with a rhythm of 2:1. This rhythm continued, the rate of the auricular beats remaining between 97 and 100 for about one hour (120 minutes after the injection); the conduction time varied between 0.11 and 0.16 of a second.

Summary.—When the dog was intact, morphin produced an arhythmia. It was characteristic of the group in which the right vagus nerve was stimulated. Ether anesthesia destroyed the arhythmia, which had partly disappeared. Soon after the end of the experiment, in which the right vagus nerve was excised, an arhythmia again appeared, which was characteristic of those observed when the left vagus nerve was stimulated.

Experiment 719.—Left vagus dog; female.

June 9, 1913. Weight 9.1 kilos. The rate was 100 and the conduction time 0.12 to 0.14 of a second. Morphin sulphate, 32 mg., was injected into an ear vein. In 1 minute the rate rose to 245.6 and the conduction time fell to 0.08 of a second. In 3 minutes the rate was 112.6 and the conduction time 0.12 of a second. One blocked auricular beat appeared. In 15 minutes the auricular rate fell to 64.2 and the ventricular to 53.8. In the sequential cycles the conduction time was 0.12 to 0.18 of a second. There were groups of left ventricular beats dissociated from the auricular contractions. Ether was administered. The rhythm became normal, though the conduction time remained high, 0.16 of a second. The rate was 97.4. One minute after the end of the operation (40 minutes after the injection of morphin) in which 3 cm. of the right vagus nerve were excised, the rate was 120.8 and the conduction time 0.1. 50 minutes later (90 after the injection) the rate was 114.8 and the conduction time 0.12 to 0.16. Blocked auricular beats had been seen for some time, but one was now recorded. The rhythm was one of incomplete auriculoventricular dissociation, the ratio being 2:1. The number of blocked auricular beats increased and records of the arhythmia were obtained for 90 minutes (180 minutes after the injection). The rate of the auricular beats varied between 107.1 and 120.8 and the conduction time between 0.1 and 0.18 of a second. The experiment was then discontinued.

Summary.—Morphin produced in the intact dog a characteristic arhythmia. It resembled certain instances in which the left vagus nerve was stimulated. Anesthesia destroyed the arhythmia. After the end of the operation, an arhythmia reappeared, recognized as typical of those produced when the left vagus nerve is stimulated.

Experiment 720.—Left vagus dog; male.

June 10, 1913. Weight 7.8 kilos. The rate was 85.7 and the conduction time 0.12 to 0.14 of a second (figure 19). Morphin sulphate, 32 mg., was injected into an ear vein. In 1 minute the rate was 200.8 and the conduction time 0.1. In 3 minutes the rate fell to 48 and the conduction time was 0.12 to 0.13 of a second. In 5 minutes there were small groups of sequential cycles at the rate of 109.8, showing a conduction time of 0.14 of a second, but the predominant rhythm was one of complete auriculoventricular dissociation, in which both auricles and ventricles beat at a rate of 34.5. Some of the ventricular beats arose in the right and others in the left ventricular wall. In 7 minutes the rate of the auricular

beats was 30.3 and that of the ventricular beats 43.7. A few sequential cycles showed a conduction time of 0.13 to 0.15 of a second. All the ventricular beats arose in the left ventricle (figure 20). Under ether anesthesia, which lasted 20 minutes (from 14 to 34 minutes after injection), the auricular rate first fell to 20 and then rose to 65.7. The ventricular rate fell correspondingly to 36.5 and rose to 72.6. A few sequential cycles were recorded during this time, showing a conduction time varying between 0.16 and 0.22 of a second. But with these few exceptions, all the ventricular beats were independent, dissociated from the auricular systoles, and arose, some in the right and others in the left ventricle. During the operation, 1.5 cm. of the right vagus nerve was excised. Directly after the end of the operation (36 minutes after injection) the rhythm was normal, the rate was 190.6, and the conduction time 0.11 of a second. 10 minutes after the operation (44 minutes after injection) the rate was 141.1 and the conduction time 0.14 to 0.16. There was now incomplete auriculoventricular dissociation, the ratio being 2:1. This rhythm, varying between 2:1 and 3:1 (figure 21), was observed for 2 hours and 15 minutes (until 179 minutes after the injection). The rate for the first hour (44 to 94 minutes after the injection) varied between 126.3 and 136.3, and the conduction time between 0.11 and 0.18 of a second. When the experiment was discontinued, the rate was 117.7, the conduction time 0.13 to 0.22. In a short strip of curve, there were twelve examples of a 2:1 rhythm and two of 3:1.

Summary.—In an intact dog morphin produced a characteristic rhythm, resembling that resulting from the stimulation of the right vagus nerve. Anesthesia destroyed this arhythmia. Soon after the end of the operation an arhythmia appeared again, but it was one characteristic of those produced when the left vagus nerve is stimulated.

Experiment 721.—Right vagus dog; female.

June 11, 1913. Weight 10.4 kilos. Morphin, 32 mg., was injected, but no arhythmia was produced.

June 13. The rate was 230 and the conduction time 0.09 of a second. Morphin sulphate, 24 mg., was injected into an ear vein. In 12 and 25 minutes two additional injections, each of 24 mg., were made (total 72 mg.). In 55 minutes after the first injection the rate was 48.7 and the conduction time 0.14. There occurred in the intervals before delayed auricular systoles a number of left ventricular beats. In 58 minutes the rate of the auricular beats was 51.08 and that of the ventricular beats 58.9. There were many left ventricular beats. Ether was administered and the arhythmia disappeared. The rate was 102.4 and the conduction time 0.13. In the operation 2 cm. of the left vagus nerve were excised. The rhythm remained normal, even though morphin, 40 mg., was injected 115 minutes after the first injection (23 minutes after the end of the operation). The experiment was discontinued, the rhythm still being normal 60 minutes later.

June 23. Second experiment. The rate was 232.3 and the conduction time 0.08 of a second. Morphin sulphate, 32 mg., was injected in an ear vein. In 3 minutes the rate was 163.3 and the conduction time 0.09 of a second. In 22 minutes morphin sulphate, 32 mg., was injected again and in 53 minutes (after the first injection) the rate was 49.6 and the conduction time 0.12 to 0.14. In 58 minutes morphin, 32 mg., was injected again. An arhythmia was now pro-

TABLE II.

Dog 704. January 31, 1913. Analysis.

		Rate 93.6	P–R = 0.1	
11.35	A.M.	Morphin 24 mg.		
11.35½	A. M.	Rate 73.8	P–R = 0.1–0.11.	
11.38	A. M.	Morphin rhythm.		
11.40	A. M.	Morphin rhythm As = 28.9	Vs = 60	P–R (in sequential cycles) = 0.12–0.14.
		Ether.		
11.50	A. M.	As = 51.8	Vs = 77.6	P–R (sequential cycles) = 0.17–0.19; both right and left ventricular systoles are present.
		Skin incision.		
11.56	A. M.	Rate 110.1	P–R = 0.13	Normal rhythm; marked changes in P waves.
		Ether stopped.		
12.02	P. M.	Right vagus cut.		
12.06½	P. M.	Operation ended.		
12.07½	P. M.	Rate 150.0	P–R = 0.1 –0.11.	
12.35	P. M.	Rate 96.8	P–R = 0.12–0.14–0.16–0.18.	
12.38	P. M.	Rate 120.0	P–R = 0.12–0.17	2 P′, progressive lengthening in antecedent P–R time.
12.52	P. M.	Rate 106.1	P–R = 0.12–0.16	5 P′.
1.02	P. M.	Rate 94.2	P–R = 0.12–0.16	8 P′.
1.45	P. M.	Rate 91.5	P–R = –0.17	6 P′.
2.09	P. M.	Rate 95.5	P–R = 0.11–0.18	5 P′.
		Morphin 24 mg.		
2.17	P. M.	Rate 114.2	P–R = 0.12–0.14	No P′.
2.18	P. M.	Rate 90.0	P–R = 0.12–0.16	4 P′.
2.25	P. M.	Rate 91.3	P–R = 0.13–0.16	10 P′.
2.40	P. M.	Rate 97.8	P–R = 0.13–0.16	5 P′.
2.25	P. M.	Morphin 24 mg.		
2.53	P. M.	Rate 93.3	P–R = 0.14–0.15	No P′.
3.00	P. M.	Rate 94.6	P–R = 0.17–0.18	7 P′.
		Morphin 128 mg.		
3.22½	P. M.	Rate 124.3	P–R = 0.1 –0.11.	
3.50	P. M.	Rate 107.3	P–R = 0.11.	

duced. In 73 minutes the rate of the auricular beats was 36.3 and that of the ventricular beats 46.4. In the sequential cycles the conduction time was 0.11 to 0.12 of a second. There were numerous left ventricular beats, independent of the auricular systoles. In 128 minutes the rhythm was normal.

Summary.—In the intact dog, an arhythmia characteristic of morphin intoxication was produced. It was characteristic rather of the rhythms produced when the right vagus nerve is stimulated. Ether anesthesia destroyed the arhythmia. Part of the left vagus nerve was excised. A later injection of morphin produced an arhythmia recognized as typical of those seen when the right vagus nerve is stimulated.

SUMMARY.

The effect of injecting morphin in right vagus dogs is to slow or practically to stop auricular systoles, the circulation being maintained by ectopic, independent, ventricular contractions. The function of conduction is, relatively speaking, undisturbed. The fact that after morphin injections there is little or no disturbance of conduction in right vagus dogs and a profound one in left vagus dogs may be used as a factor in distinguishing between them, especially when there is a reduction in rate greater than usual in left vagus dogs. The effect on left vagus dogs is to slow the rate of the auricles moderately and to increase the length of conduction, so that partial auriculoventricular block or complete auriculoventricular dissociation results. These are precisely the results that have been obtained by faradic stimulation of one vagus nerve, the other being divided. There are slight differences in the results obtained between the two methods, but the explanation for these is probably to be found in the differences in the nature of the experiments. When faradic stimulation is employed, the stimulus is applied a short time only,—in our experiments for periods not longer than ten seconds. The occurrence of the maximum effect is sudden and does not provide for a gradual introduction of the ventricles to new conditions. In right vagus stimulation the ventricles usually stop beating. A gradual introduction is, however, not always necessary, as may be seen in figures 5 and 6 of the paper (4) already quoted, where idioventricular rhythms began without delay. Another difference between the two methods is found in the degree of effect produced on stimulating the left vagus; in the faradic method the change in conduction varies from mere lengthening to a condition so profound that complete dissociation results; these degrees have been described. The mor-

phin method usually produces only incomplete dissociations. Twice only was complete dissociation produced.

The similarity between the results of the two methods is sufficiently close to render it likely that in obtaining them an identical mechanism in the heart is involved. It may, therefore, be concluded that the inconstant action of the vagus resulting from morphin injections, called "*ungleichartig*" by Einthoven and Wieringa, appears so on account of the fact that the predominating effect of morphin may be exercised now on one and now on the opposite cardio-inhibitory system, and not, as was suggested, on account of a shifting of predominance from one to another of the fiber tracts in the vagi themselves. The explanation offered as the results of this series of experiments differs from theirs. The results obtained substantiate the conclusion reached in the former series, that the two vagi act differently. A fact relating to the mechanism of the right vagus nerve can now be added, from a consideration of the cardiac mechanism in right vagus dogs, namely, that derangements in conduction result, to a very slight extent only, as the effect of the influence of the right vagus nerve

Although no solution of the production of the extreme grades of sinus irregularity seen in morphin intoxication is offered, the failure of this rhythm to be converted into a more profound irregularity may be explained. There seems little doubt that the occasion for the occurrence of an independent ventricular rhythm in these experiments was due to the slowness of the auricular rate resulting from morphin injection. But in the cases of sinus irregularity (Nos. 683, 685, and 700), the rate of the auricles was never sufficiently low for the ventricles to initiate an independent rhythm. The lowest rates recorded in them were 66.3 (No. 683), 85.7 (No. 685), and 91.4 (No. 700), and it appears that before an independent ventricular rhythm occurred in any of the experiments, the auricular rate had always fallen below 41 (Nos. 686, 688, 697, 698, 706, 715, and 721). On the other hand, the ventricular rates in the cases of sinus irregularity were higher than the highest idioventricular rates observed and no advantage could consequently have been gained by the onset of a new rhythm. No. 683 was an exception, but the rate in this case exceeded the idioventricular rates of all

but Nos. 702 and 706 and was only 11.1 beats below that of the highest recorded. Whether sinus irregularity itself is a morphin irregularity the result of a sino-auricular block, in the sense of Eyster and Meek, has already been discussed.

CONCLUSIONS.

From a consideration of the facts described in these experiments, the following statements are justified:

1. Morphin, when injected intravenously into dogs, causes a disordered rhythm, resulting from its action on the inhibitory apparatus. The arhythmia exhibits characteristics of both right and left vagus stimulation, and combinations of the two.

2. In dogs with a right vagus nerve only, the rhythm consists in a decided slowing of the rate of the auricles, and the development of an idioventricular rhythm.

3. In dogs with a left vagus nerve only, the rhythm consists in moderate or no slowing of the auricular rate, and marked disturbance of conduction which sometimes leads to the occurrence of incomplete auriculoventricular block (2:1 and 3:1). The disturbance in conduction is sometimes so great that no auricular impulses reach the ventricles, and these then develop their inherent rhythm in complete dissociation from the auricles.

4. A rhythm called a sinus irregularity also occurs. The mechanism producing this is not fully explained.

5. (*a*) The similarity of the rhythms produced by morphin in right vagus dogs and those caused by stimulating (electrically) the right vagus nerve, when the other vagus is cut, is complete.

(*b*) The similarity of the rhythms produced by morphin in left vagus dogs and those caused by stimulating (electrically) the left vagus nerve, when the other vagus is cut, is also complete.

6. The disorder in the cardiac mechanism, described by Einthoven and Wieringa, finds a complete and simple explanation. In their experiments morphin acted either alternately on both inhibitory mechanisms or on the two in combination.

7. This series of experiments substantiates, by a method under conditions more nearly normal, the conclusions reached in a former

paper, wherein similar differences between the vagus nerves are described.

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EXPLANATION OF PLATES.

In all the figures there are shown an electrocardiogram taken from the right fore and left hind leg, a time curve marked in 0.02, and one in 0.2 of a second.

PLATE 69.

FIG. 1. Dog 702. Taken before the injection of morphin. It serves as a control.

FIG. 2. Dog 702. Taken after the injection of morphin. Three left and three right ventricular complexes are seen, and between them one intermediate in form between the two. No auricular complex is identified in the portion of the curve reproduced; a comparison with figure 3 makes it probable that none occurred. The ventricular rate is 63.3, the auricular is 5.

FIG. 3. Dog 702. Taken after the right vagus nerve was frozen. The auricular rate is 88.8 and the ventricular 89.6. There is complete auriculoventricular dissociation. The third ventricular complex is deformed by an auricular deflection. It is clear that the other complexes are pure.

FIG. 4. Dog 685. Taken during the release (by atropin injection) from slow sinus irregularity caused by morphin. Incomplete auriculoventricular block (2:1 and 3:1) is shown.

FIG. 5. Dog 686. Taken after the injection of morphin in a right vagus dog. The auricular rate is 32.3, the ventricular is about 48. The conduction time in the sequential cycles is 0.11 to 0.12 of a second. Part of the curve shows an auriculoventricular dissociation due to the slow auricular rate; the ectopic ventricular systoles are not premature. There is no defect in conduction as shown by the sequential cycles.

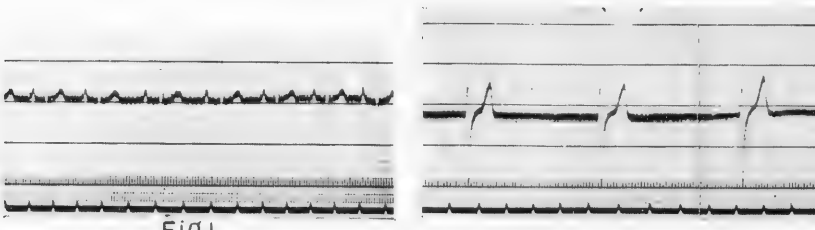


Fig. 1.

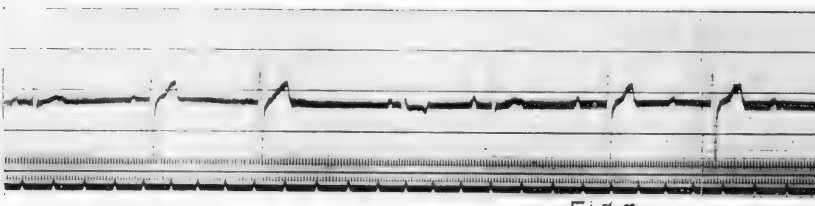


Fig. 3.

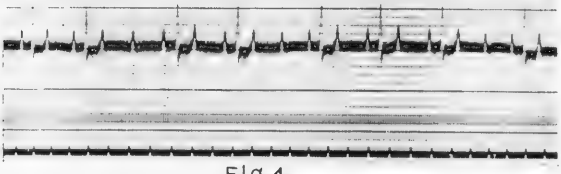


Fig. 4

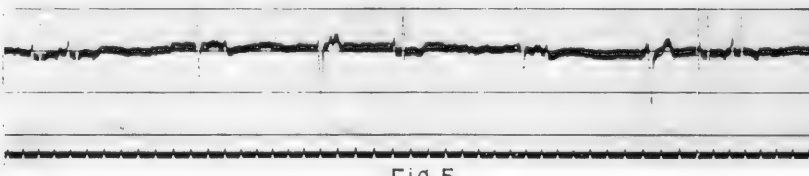


Fig. 5.

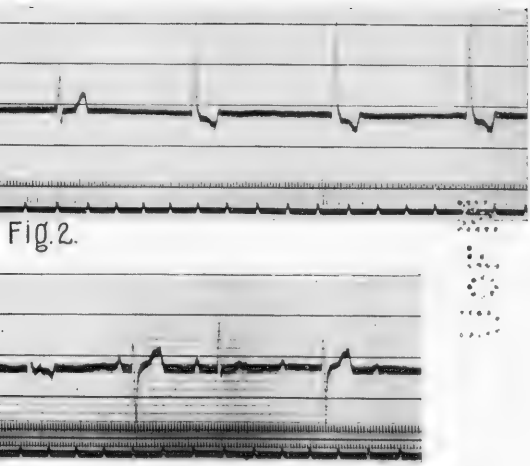
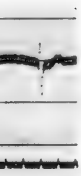


Fig. 2.



(Cohn: Effect of Morphin on Dog's Heart.)

PLATE 70.

FIG. 6. Dog 688. Jan. 9, 1913. Taken after the injection of morphin in a right vagus dog. The auricular rate is 49. There is marked sinus irregularity. In the long interauricular pauses, ectopic ventricular contractions occur. Two of these are shown; the first is pure, the second deformed by an auricular beat. The conduction time in the sequential cycles is 0.11 to 0.13 of a second.

FIG. 7. Dog 688. Feb. 14, 1913. Taken before the injection of morphin. It serves as a control. The rate is 127.2 and the conduction time 0.11 to 0.12 of a second.

FIG. 8. Dog 688. Taken 22 minutes after the injection of morphin. The auricular rate is about 11, the ventricular 57. The positions of the auricular representatives are indicated by the asterisks. The ventricular complexes with which these deflections are associated do not show the small downward deviation seen at the beginning of the T wave of the other complexes. These small waves represent auricular beats due to retrograde stimuli from the ventricles. One sequential cycle is shown.

Fig. 9. Dog 688. Rates similar to those seen in figure 8. The position of the auricular wave expected between the two at the ends of the curve is indicated by the anomalous shape of the ventricular complex marked * and by the absence of the downward deflection at the beginning of the T wave in this complex. A similar complex is recognized in the one before the last.

FIG. 10. Dog 698. Taken before the injection of morphin. The rate is 95.9 and the conduction time 0.12 of a second. It serves as a control.

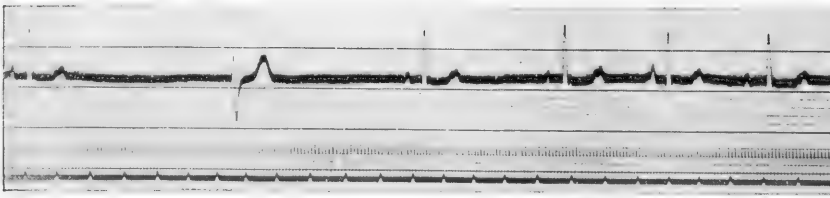


Fig. 6.

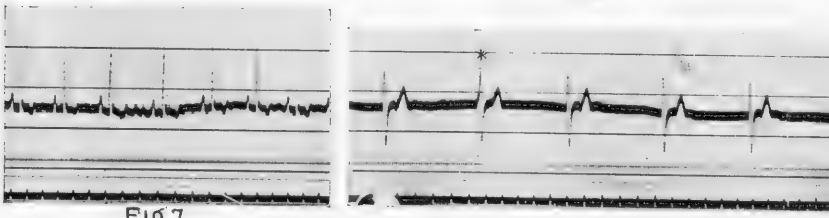


Fig. 7

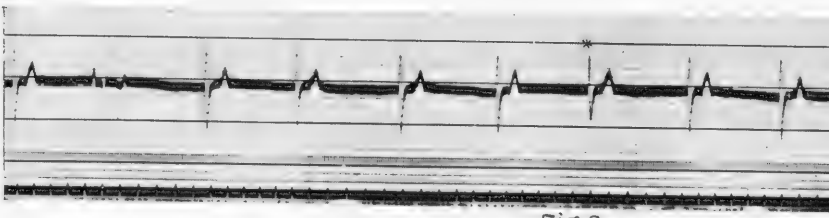
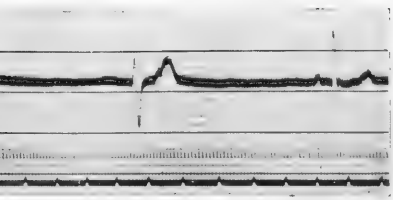
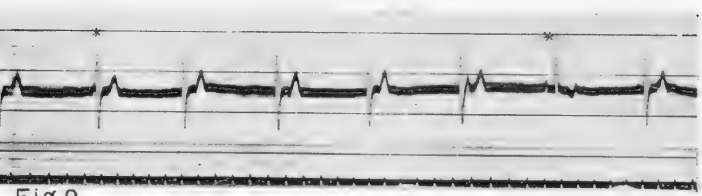


Fig. 9.





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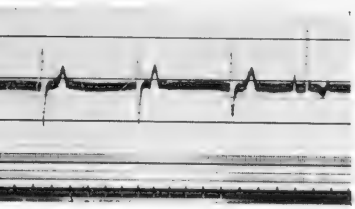


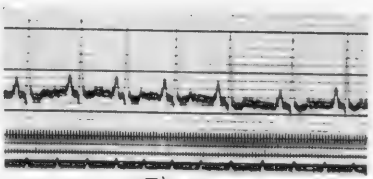


Fig.10.

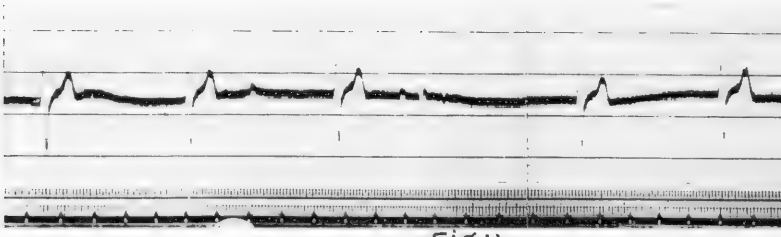
(Cohn: Effect of Morphin on Dog's Heart.)

PLATE 71.

FIG. 11. Dog 698. Taken 13 minutes after the injection of morphin in a right vagus dog. The auricular rate was about 24, the ventricular about 66. The ventricular beats are ectopic, except two sequential cycles which have a conduction time of 0.13 to 0.15 of a second.

FIG. 12. Dog 698. Taken 22 minutes after the injection of morphin. No auricular representatives are seen in the curve. The ventricular rate is 64. A comparison of this with the other curves from this experiment suggests the undeformed nature of these complexes.

FIG. 13. Dog 698. Taken 44 minutes after the injection of morphin. The ventricular rate is 69. Auricular representatives are seen occurring at the same rate; they are inverted waves and are probably dependent on the ventricular complexes.



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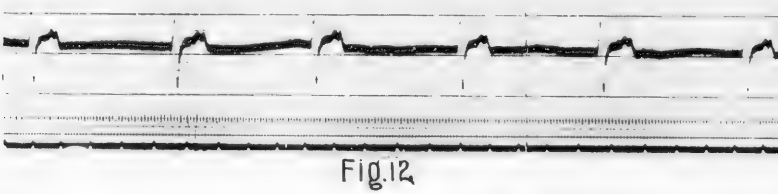

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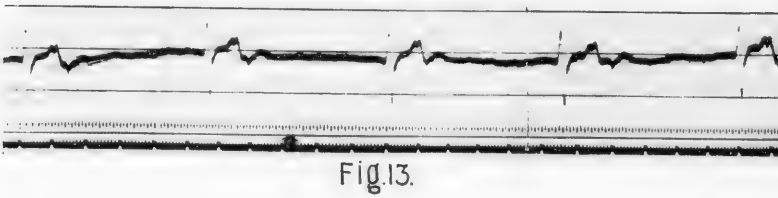
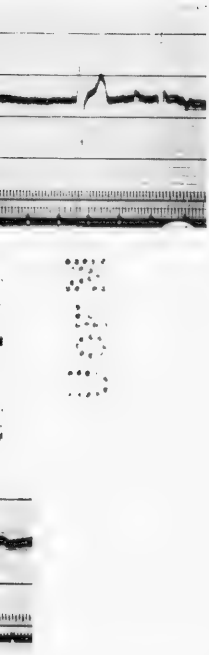


Fig. 13.

PLATE 71.



Morphin on Dog's Heart.)

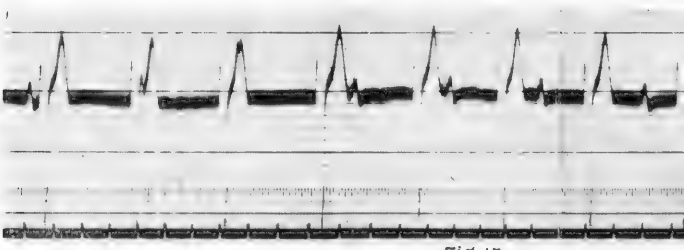
PLATE 72.

FIG. 14. Dog 715. Taken before the injection of morphin. The rate is 126.5 and the conduction time 0.14 of a second. It serves as a control.

FIG. 15. Dog 715. Taken during anesthesia before excision of the vagus nerve. It shows complete auriculoventricular dissociation. The independent ventricular beats are ectopic.

FIG. 16. Dog 715. Taken about 9 minutes after figure 15. It shows the transition from complete dissociation to a sequential rhythm with prolonged conduction time (0.21 of a second).

FIG. 17 Dog 715. Taken several days later, 24 minutes after the injection of morphin in a right vagus dog. The auricular rate is about 30, the ventricular 67. The ventricular contractions are ectopic. The sequential beats show a conduction time of 0.16 to 0.18 of a second.



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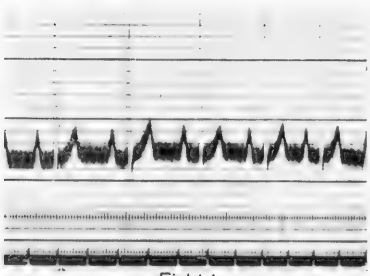

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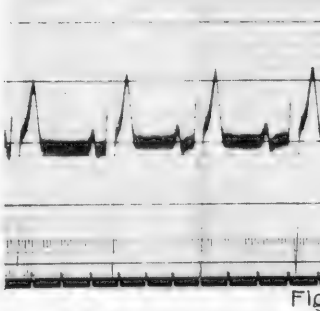

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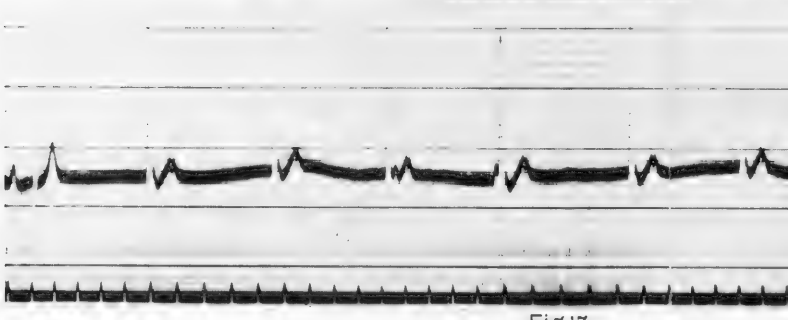
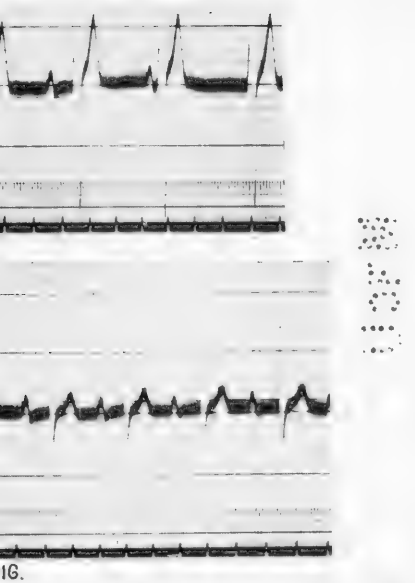
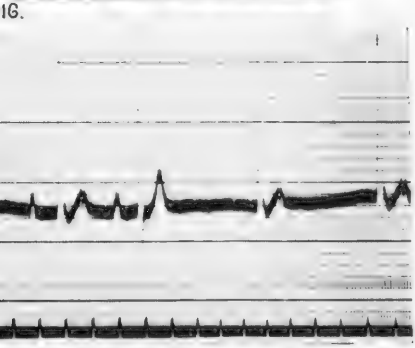


Fig. 17.



16.



(Cohn: Effect of Morphin on Dog's Heart.)

PLATE 73.

Fig. 18. Dog 715. Taken one minute after figure 17. Five auricular beats (the first two not reproduced) occurred in rapid succession. The three beats shown form sequential cycles and have a conduction time of 0.14 to 0.16 of a second. No other auricular representatives are seen in the rest of a long curve (15 seconds). The ventricular rate is 61.9.

FIG. 19. Dog 720. Taken before the injection of morphin. The rate is 85.7 and the conduction time 0.12 to 0.14 of a second. It serves as a control.

FIG. 20. Dog 720. Taken 7 minutes after the injection of morphin before the vagus nerve was excised. The auricular rate is 30, the ventricular 43.7. The two sequential cycles shown have a conduction time of 0.13 and 0.15 of a second. The ventricular complexes are ectopic and dissociated from the auricular ones.

FIG. 21. Dog 720. Taken 86 minutes after morphin injection in a left vagus dog. The auricular rate is 130. Four examples of incomplete heart-block at a ratio of 2 : 1 and one at 3 : 1 are shown.

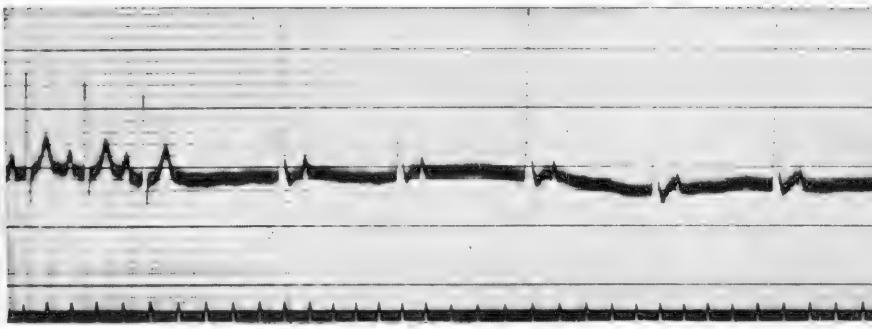


Fig. 18.

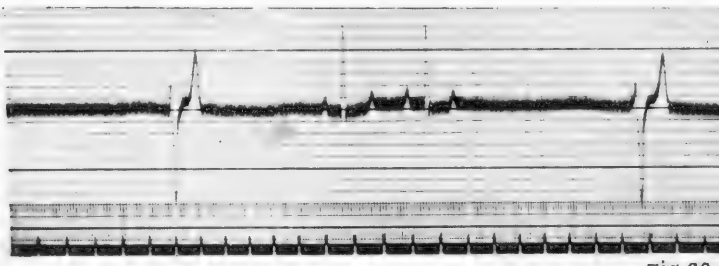


Fig 20.

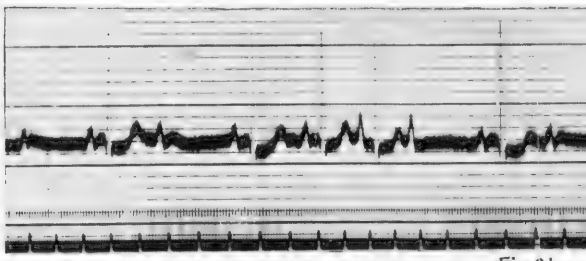


Fig. 21.

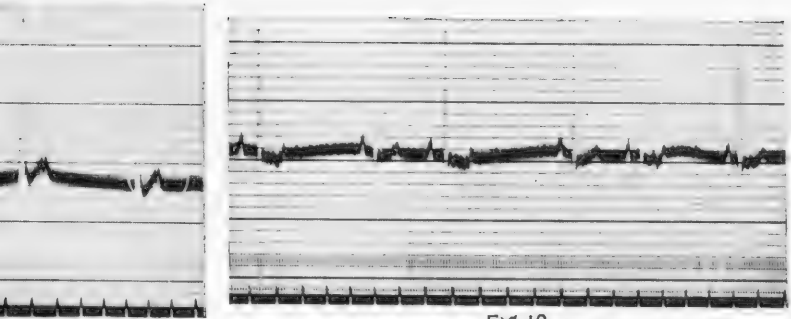
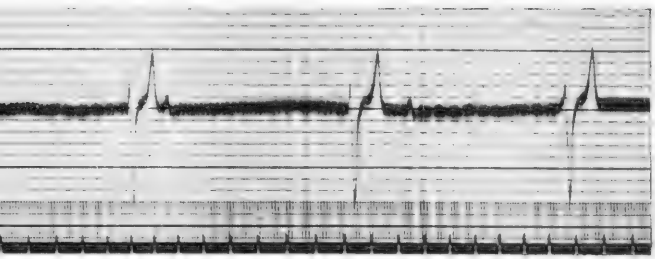
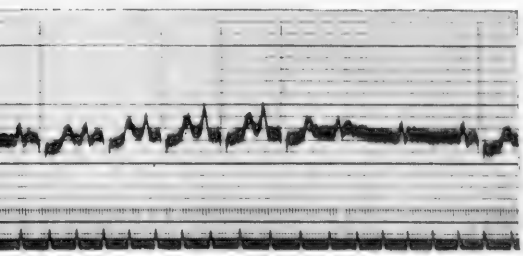


Fig. 19.





(Cohn: Effect of Morphin on Dog's Heart.)

THE PREDOMINANT INFLUENCE OF THE LEFT VAGUS NERVE UPON CONDUCTION BETWEEN THE AURICLES AND VENTRICLES IN THE DOG.*

By ALFRED E. COHN, M.D., and THOMAS LEWIS, M.D.

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PLATE 74.

It has been shown by one of us (1) that the normal production of impulses in the region of the sino-auricular node is inhibited by stimulation of the right vagus nerve to a greater extent than by stimulation of the left. Earlier observations, which are fully discussed in that paper, had indeed indicated that such might prove to be the case; but a clear demonstration of the preponderating influence of the right vagus upon the sino-auricular node, in which structure the normal impulses are almost certainly generated, had not been published.

In the same paper, as in the discussed observations of previous workers, it was stated that the left vagus had an influence upon the tissues uniting auricle and ventricle which was not obtained on stimulating the right vagus nerve, stimulation of the left vagus nerve producing high grades of heart-block. A comparison of the right and left vagi in this respect has not been possible in previous experiments, for the reason that the right vagus slows the auricle to a greater extent than does the left. It seemed possible to us that the profound effect of left vagal stimulation upon conduction over the A-V junction, and the absence of such effect or the observation of a lesser effect upon stimulation of the right vagus upon the same region of the heart, may have been due to the usual difference in the retardation of auricular rate. For we know that auriculoventricular heart-block is favored by high auricular rate (2 and 3). We

* Dr. Lewis's expenses have been defrayed by grants from the Graham Research Fund and the Royal Society. The observations were undertaken in London. Received for publication, September 25, 1913.

therefore determined to examine the influence of right and left vagal stimulation upon A-V conduction while the auricular rate was maintained artificially at a constant level.

METHOD.

Dogs[1] were employed exclusively and were anesthetized with morphia, paraldehyde, and ether. Records of the heart beat were taken electrocardiographically, lead II being utilized. The cut vagi have been stimulated by means of a faradic current, the primary circuit of the inductorium being excited at a constant voltage, the secondary coil being placed at fixed points, and the leads from secondary coil to right and left nerves, running through a simple mercurial commutator, having equal resistances. The right and left vagus nerves have been stimulated alternately, usually the right nerve first, but from time to time the left. Sufficient time for complete recovery elapsed between successive stimulations. On occasion we reversed the positions of the stimulating electrodes, but our results remained the same.

The auricles were controlled by rhythmic induction shocks, at or a little above threshold value, applied through a window in the chest wall to the right auricular appendix or to the area surrounding the sino-auricular node; we chose this area for stimulation so as to ensure auricular complexes of identifiable form.

RESULTS.

The detailed results of seven experiments are given in the following tables. Each experiment includes a number of observations. The effects of right and left vagal stimulation are expressed in the end columns: P-R + signifies a prolongation of conduction interval; 2:1, 3:1, 4:1, etc., expresses the ratio of auricular and ventricular rates and the degree of heart-block; $2:1^3$ indicates the occurrence of three 2:1 cycles; A. F. indicates the onset of auricular fibrillation sometimes seen upon stimulation; "slight slowing" refers to slowing of the whole heart without alteration of sequence. Each experiment is summarized at the end of the corresponding section of the table. The first column tells whether the auricles were artificially maintained at a constant rate of action ("As stim."), or

[1] The operative procedures were undertaken by Lewis.

whether the observation consisted of simple vagal stimulation ("control"). The rate of the auricles, under one or the other condition, is given in the second column. The remaining columns refer to the length of stimulation and the position of the secondary coil.

We may briefly sum up our results in a second table (table II). In five out of seven experiments, simple stimulation of the right and left vagus ("controls") yielded the customary effects and characteristic differences of effect. The right vagal stimulation yielded profound slowing of the whole heart,[2] or stoppage of the auricle with escape of the ventricles; the left vagal stimulation a moderate degree of auricular retardation with the development of partial heart-block. In two experiments (dogs DU and DW) right and left stimulations yielded, in the control observations, simple slowing of the whole heart, in one case the effect of the left nerve being the greater, in the other case the effects being equal on the two sides and being accompanied by an equal increase of the conduction interval.

TABLE I.

Dog DS. August 5, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
1. Control..	105.0	Left	6.2	23		P−R++, 4:1.
2. Control..	99.0	Right	5.2	23	As stopped; 1 Vs escaped	
3. As stim..	194.0	Left	5.0	23		19:1, P−R++.
4. As stim..	194.0	Right	5.0	23	15:1, 2:1[3], P−R++	
5. As stim..	192.0	Left	4.6	25		P−R+, 1:1.
6. As stim..	192.0	Right	5.4	25	No effect	
7. As stim..	143.1	Left	5.2	25		P−R++, 4:1[2], 2:1[2].
8. As stim..	137.0	Right	6.0	20	P−R++, 2:1[4]	
9. As stim..	138.4	Right	5.3	25	P−R++, 2:1[3], A.F.	
10. As stim..	138.4	Left	5.7	25		P−R++, 1:1.

Summary.—The control stimulations showed the usual differences between stimulation of the right and left nerves. With the auricular rate maintained, the left gave a higher grade of block than the right on two occasions, the same grade on one, and a lesser grade on one occasion.

[2] With or without slight prolongation of the conduction interval.

Dog DT. August 11, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
1. As stim...	No time marker	Right	5.0	20	3:1, 4:1^2, 3:1, 2:1	
		Left	5.0	20		4:1, 2:1^2, 4:1, 2:1^2.
2. As stim...	No time marker	Right	5.0	20	3:1, 2:1, 3:1^3, 2:1	
		Left	5.0	20		2:1, 3:1, 1:1, 2:1.
3. As stim...	210.0	Left	4.8	20		3:1, 5:1, 4:1, 2:1^2.
	210.0	Right	5.5	20	3:1, 4:1, 13:1 (2RVs), 2:1	
4. Control...	180.0	Left	4.5	20		2:1^3.
	175.6	Right	5.2	20	3P′+3RVs	
5. As stim...	210.0	Left	5.3	22		P−R slightly+.
	195.6	Right	5.2	22	P−R+	
6. As stim...	219.0	Left	4.8	20(?)		P−R+, 2:1.
	217.0	Right	5.2	20(?)	P−R+, 2:1^2, 1:1, 2:1^2	
7. As stim...	212.2	Right	4.9	21	5:1, 4:1^2, 3:1, 2:1	
	216.6	Left	5.6	21		P−R+, 3:1, 2:1, 3:1, 2:1^3.

Summary.—Control stimulations gave usual differences in reaction to right and left vagal stimulations. With the auricular rate maintained, right vagal stimulation gave greater degrees of heart-block than the left on all six occasions.

Dog DU. August 14, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
1. Control...	184.2	Left	5.5	21		Slowing only.
	182.0	Right	4.6	21	Slowing only	
2. As stim...	228.3	Left	4.6	21		3:1, 6:1, 8:1, 1:1, 1(regular):1.
	232.0	Right	6.4	21	3:1^2, 4:1, 3:1, 10:1, A.F.	
3. Control...	201.0	Right	5.5	22	Slight slowing only	
	196.5	Left	4.9	22		Moderate slowing only.
4. As stim...	225.6	Right	5.2	21½	2:1^2, 1:1^2, 2:1, 5:1, 3:1	
	228.6	Left	5.1	21½		20:1, A.F.
5. Control...	192.0	Right	5.3	21½	Moderate slowing only	
	188.4	Left	6.2	21½		More slowing than R; no block.

Summary.—The control stimulations gave an unusual effect. Simple slowing was obtained with stimulation of both nerves, the effect being greater on the left side. The auricular rate being maintained, no difference was found in the degree of block produced by stimulating the two nerves.

Dog DV. August 15, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
1. Control...	112.5	Right	Signal not photographed	25½	Slowing only	
	114.6	Left		25½		6:1 (Vs stopped).
2. As stim...		Right	Signal not photographed	23	No effect	
		Left		23		2:1², 6:1, 5:1, 2:1² alternation.
3. As stim...	164.0	Right	5.1	22	3:1, 2:1⁵, P−R+	
	165.0	Left	5.3	22		15 : 1, 2 : 1², P−R+.
4. As stim.	189.0	Right	Only 3.6 stim. photographed	22	2:1⁶, P−R+alternation	
	192.0	Left	5.2	22		16:1, 2:1², P−R+.
5. As stim...	186.0	Right	5.9	22	P−R+, 2:1²	
Control...	163.05	Left	6.1	22		P−R+, 2:1⁴.
6. Control...	171.6	Right	5.1	21	Slowing with P−R+	
	167.4	Left	5.1	21		P−R+, 2:1⁴.
7. As stim...	192.0	Right	5.6	21	Disordered rhythm, 2:1², 4:1, 5:1, 2:1	
	194.0	Left	5.3	21		P−R+, 8:1, 4:1², 2:1².

Summary.—The control stimulations gave the usual effects. The auricular rate being maintained, left vagal stimulation produced a greater degree of heart-block than the right on all four occasions.

Dog DW. August 18, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
1. Control...	156.0	Right	5.7	22	Slowing with P−R slightly +	
	154.5	Left	5.5	22		Slowing with P−R slightly +.
2. As stim...		Right	5.6	22		
	192.0	Left	5.6	22		P−R++, 5:1, 12:1, A.F.
	186.0	Right	5.7	22	P−R slightly+, 5:1, 4:1, 6:1, 2:1, 4:1³, 2:1	
3. As stim...	188.4	Right	6.1	22	6:1, 12:1, 2:1	
	184.5	Left	6.2	22		8:1, 12:1, A.F.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
4. As stim...	186.0	Right	6.2	22½	P−R+, 2:1², 1:1², 2:1³	P−R++, 2:1⁴, 4:1, 2:1.
	186.0	Left	5.6	22½		
5. Control...	156.3	Right	5.6	22½	Slowing with P−R slightly +	
	159.0	Left		22½		Slowing with P−R slightly +.
		Left	6.3	22½		
6. As stim...	180.0	Right	5.3	22½	P−R+, 2:1², 1:1, 2:1²	P−R++, 2:1⁶.
	180.0	Left	5.8	22½		

Summary.—The control effects were unusual in type, simple slowing of equal amounts being obtained from the right and left vagi. The auricular rate being maintained, left vagal stimulation produced a greater degree of heart-block than the right on all four occasions.

Dog DX. August 21, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
1. Control...	134.6	Right	5.12	23	Slowing only	
	135.0	Left	5.14	23		3:1, 2:1², P−R+.
2. As stim...	203.4	Right	5.00	23	P−R, 2:1⁵	
	201.0	Left	4.92	23		P−R++, 17:1, 2:1.
3. As stim... (Figures 3 and 4)	195.0	Right	4.88	23	P−R+, 2:1²	
	204.0	Left	5.12	23		P−R++, 18:1.
4. Control... (Figures 1 and 2)	159.6	Right	5.36	23	Slowing only	
	156.9	Left	5.29	23		3:1, 2:1³, P−R+.
5. As stim... (Figures 5 and 6)	193.5	Right	5.40	23½	P−R slightly +, 1:1	
	188.1	Left	4.89	23½		P−R++, 5:1, 3:1, 3:1².
6. Control...	152.1	Right	5.16	23½	Slowing only	
	152.1	Left	5.14	23½		P−R+, 3:1, 2:1³.

Summary.—The control stimulation gave the usual differences on the two sides. With the auricular rate maintained, left vagal stimulation gave greater degrees of heart-block on all three occasions.

Dog DY. August 21, 1913.

Experiment.	Rate of heart.	Vagus.	Length of stimulus in seconds.	Secondary coil at	Right.	Left.
7. Control..	230.4	Right	5.6	23	Slowing with P−R slightly +	
	227.6	Left	4.72	23		P−R++, 3:1, 2:1[4].
8. As stim..		Right	5.2	23	P−R+, 4:1, 6:1, 4:1[2], 2:1[2]	
9. Control..	211.4	Right	5.2	22½	Slowing with P−R+	
	210.0	Left	5.16	22½		P−R++, 2:1[3].
10. As stim..	210.0	Right	5.32	22½	P−R+, 3:1, 2:1, 6:1, 3:1	
	210.0	Left	5.14	22½		P−R+, 4:1, 8:1, 4:1, 2:1.
11. Control..	198.0	Right	5.44	22½	Slowing with P−R slightly +	
	198.0	Left	5.56	22½		P−R+, 3:1, 2:1[4].
12. As stim .	210.0	Right	5.4	22½	P−R+, finally A.F.	
	212.0	Left	5.2	22½		P−R+, 12:1, 4:1, 2:1[2].
13. Control..	188.1	Right	4.98	22½	Slowing with P−R+	
	185.1	Left	5.4	22½		P−R+, 3:1, 4:1, 2:1[5].
14. As stim..	240.0	Right	5.92	22½	P−R+, 3:1, 6:1, 8:1, 3:1, 2:1	
	243.6	Left	5.0	22½		P−R+, 18:1 (+1 LVs), 3:1, 2:1.
15. Control..		Right	4.72	22½	Slowing with P−R+	
	173.8	Left	4.88	22½		P−R+, 9:1, 2:1.

Summary.—The control stimulations gave the usual differences on the two sides. With the auricular rate maintained, stimulation of the left vagus gave greater degrees of heart-block than the right on all three occasions.

In all the experiments, while the auricular rate was maintained at a constant level throughout an observation, profound changes in conduction were obtained, but the effects were unequal on the two sides. In all but one experiment the left vagus was the more potent in producing heart-block. In the single experiment (DT) in which the right nerve seemed more potent than the left in this respect, the difference in effect was relatively small. It is of interest to note

that in the control stimulations of this experiment, conduction effects were noted only upon stimulation of the left nerve. This experiment suffices to emphasize the impossibility of comparing the actual conduction defects produced by simple stimulation of the two nerves, and the necessity of maintaining a constant auricular rate.

TABLE II.

No. of dog.	Date.	Controls.	Effect on conduction present.		Greater effect on conduction.
			Right.	Left.	
DS	Aug. 5, 1913	Usual effects with characteristic difference	+	+	Left.
DT	Aug. 11, 1913	Usual effects with characteristic difference	+	+	Right.
DV	Aug. 15, 1913	Usual effects with characteristic difference	+	+	Left.
DX	Aug. 21, 1913	Usual effects with characteristic difference	+	+	Left.
DY	Aug. 21, 1913	Usual effects with characteristic difference	+	+	Left.
DU	Aug. 14, 1913	Simple slowing on both sides, the left being more effective	+	+	Left.
DW	Aug. 18, 1913	Simple and equal slowing on the two sides	+	+	Left.

Briefly, our results clearly indicate that both right and left vagal stimulation hinders the passage of impulses from auricle to ventricle, and show that the difference in action is quantitative rather than qualitative. The left nerve has more control over the junctional tissues than the right. Now we have seen that so far as impulse formation in the auricle is concerned, the opposite is the case, the right nerve being the more potent; yet here again the difference is quantitative rather than qualitative. We may sum up our knowledge of the vagi in the dog in the statement that while both nerves usually affect impulse formation and impulse conduction, the right nerve chiefly controls the former and the left nerve the latter.

These facts are of more interest in the light of recent physio-

EXPLANATION OF PLATE 74.

The figures are all taken from dog DX, August 21, 1913. They are from lead II. The interval between each pair of ordinates equals 0.04 of a second. The uppermost line is a signal showing the duration of the stimulus applied to the vagus nerve.

FIG. 1. Stimulation of right vagus nerve. Control.

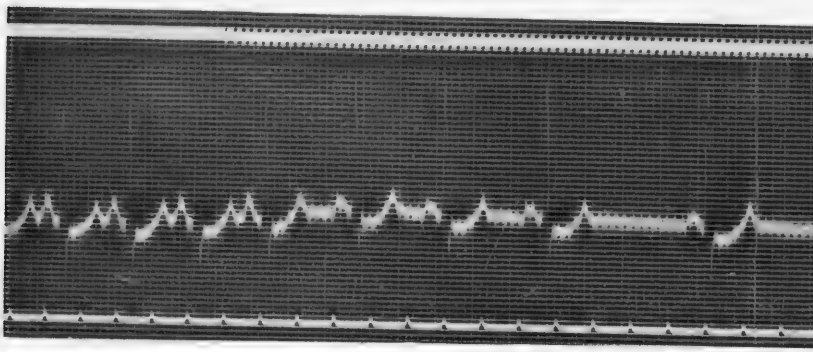
FIG. 2. Stimulation of left vagus nerve. Control.

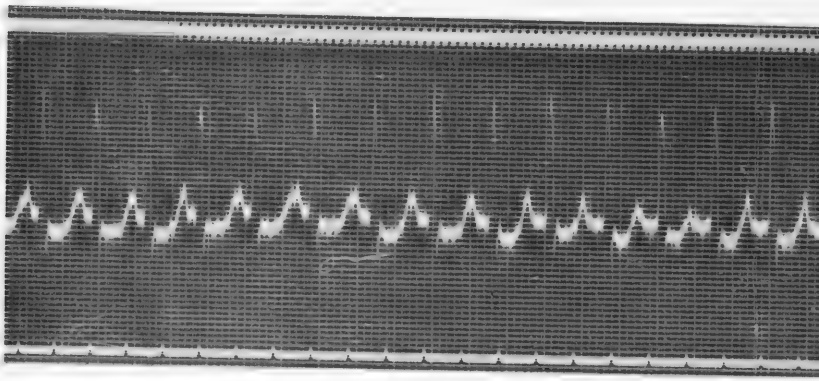
FIG. 3. Stimulation of right vagus nerve. The auricles received 195 induction shocks per minute.

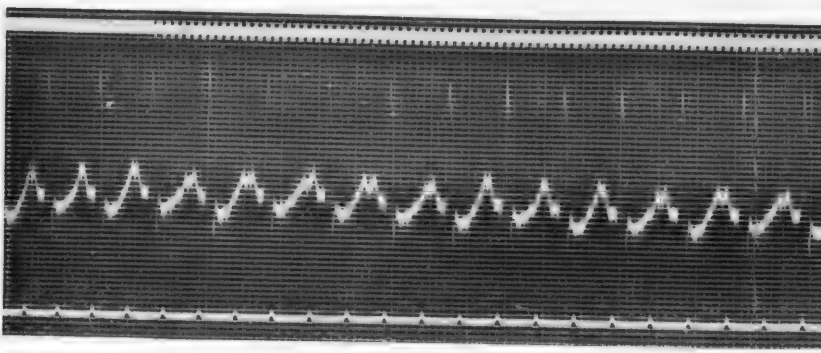
FIG. 4. Stimulation of left vagus nerve. The auricles received 204 induction shocks per minute.

FIG. 5. Stimulation of right vagus nerve. The auricles received 193.5 stimuli per minute.

FIG. 6. Stimulation of left vagus nerve. The auricles received 188.1 stimuli per minute.







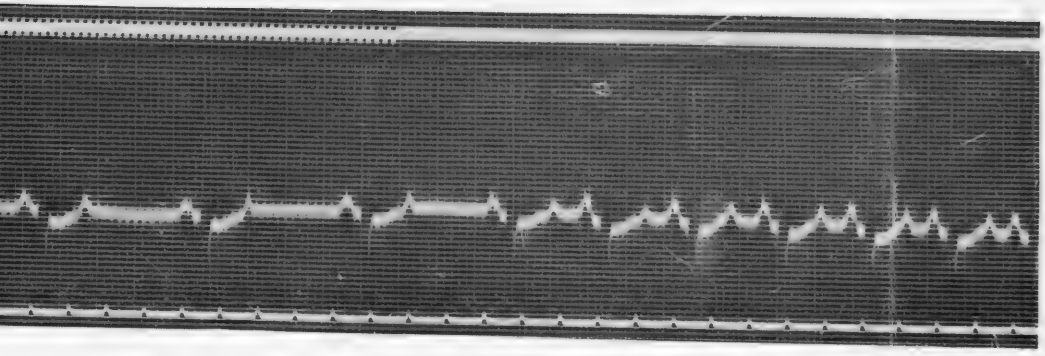


FIG. 1.

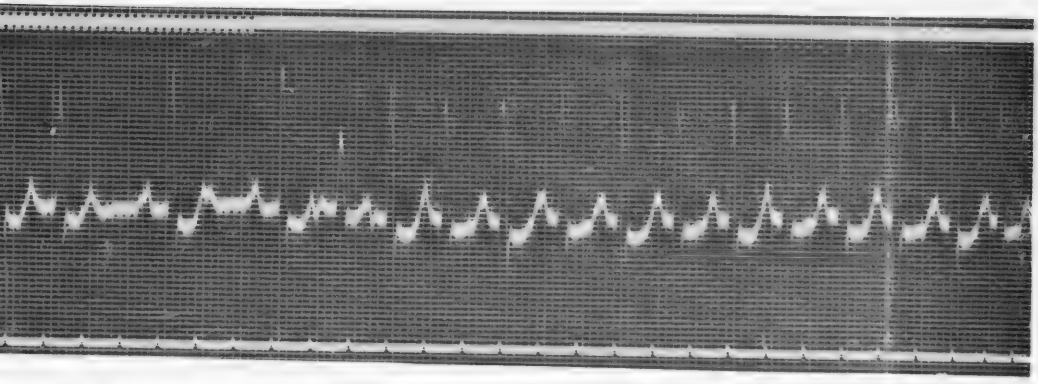


FIG. 3.

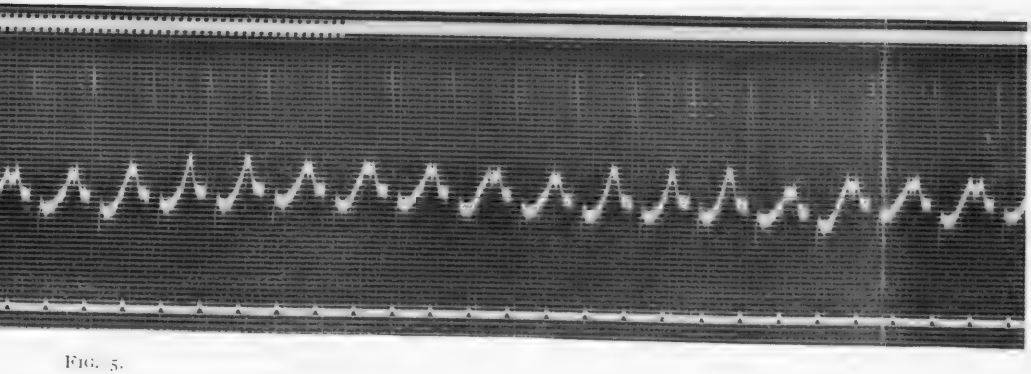
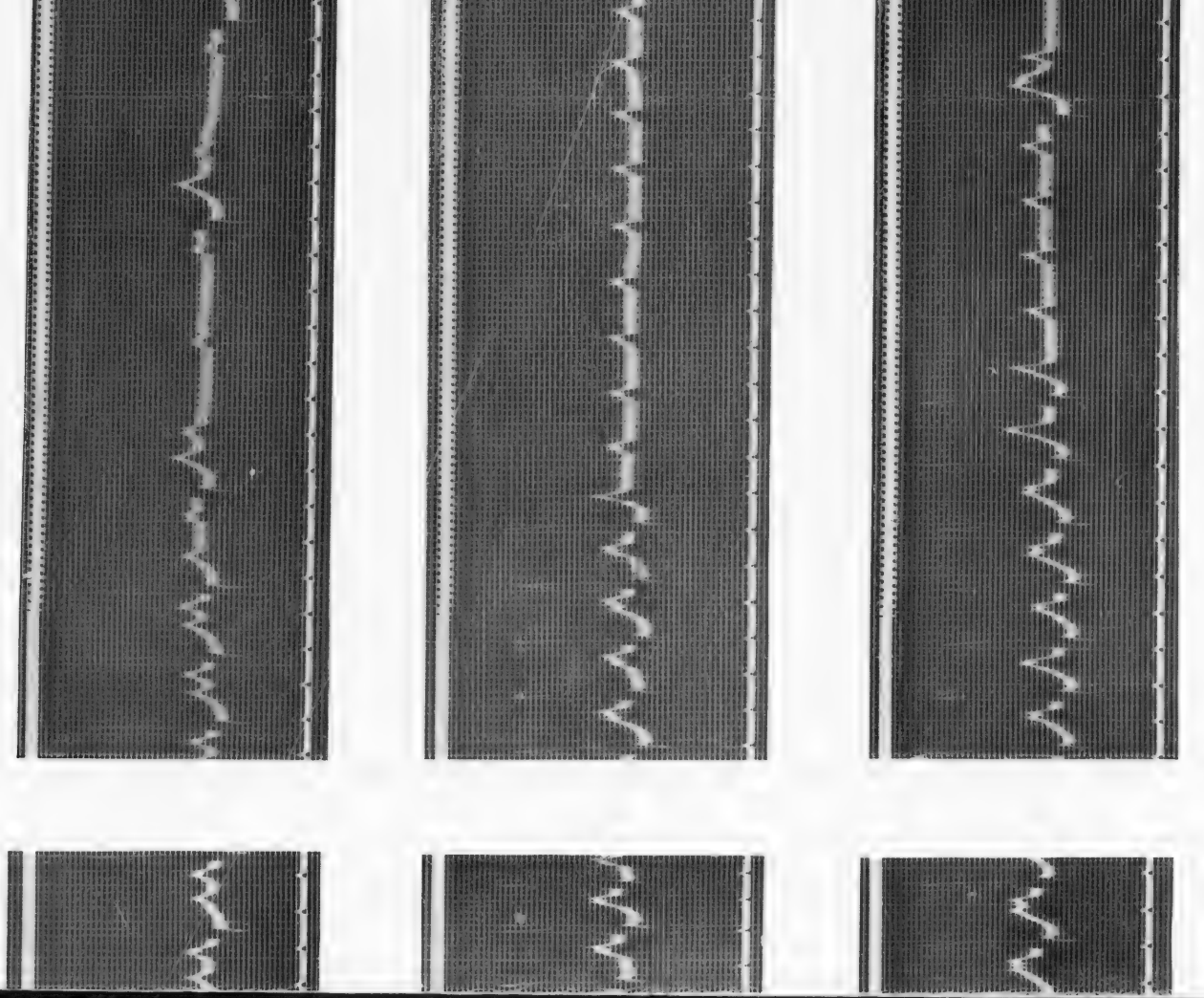


FIG. 5.



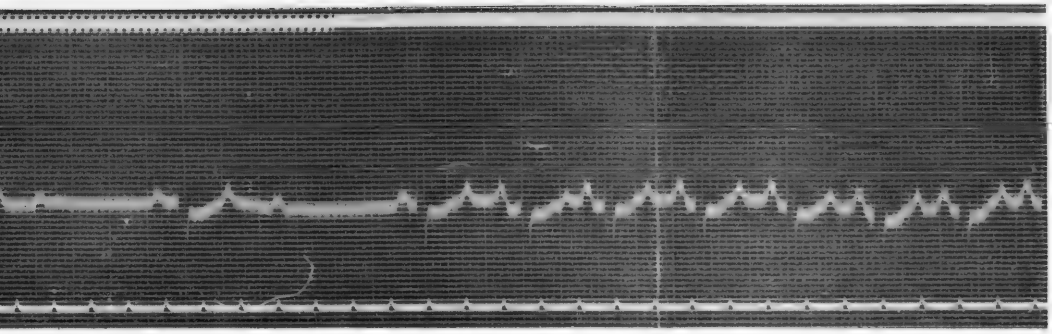


FIG. 2.

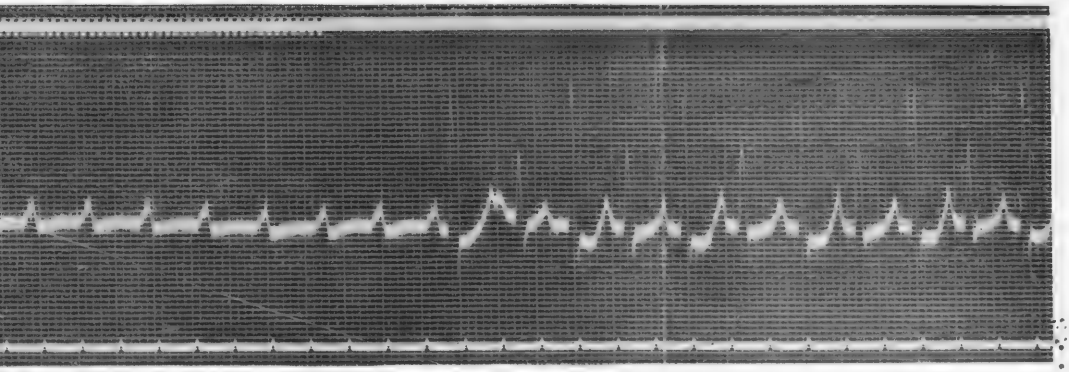


FIG. 4.

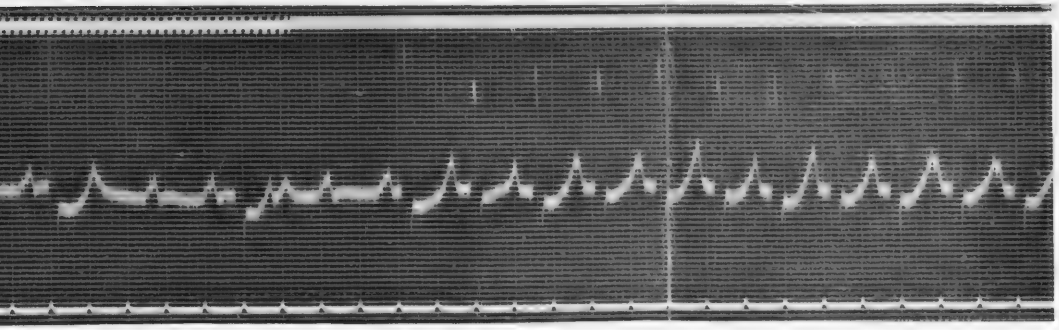


FIG. 6.

(Cohn and Lewis: Influence of Left Vagus Nerve.)

logical and morphological observations. We are taught, on the one hand, that the sino-auricular node is the site of normal impulse formation; and, on the other, that the auriculoventricular node and the tissue which joins it to the auricle is the upper end of the conduction system. These structures, situated at the mouths of the superior vena cava and at the coronary orifice (the representatives of the right and left ducts of Cuvier), have been regarded by Mackenzie (4) as homologous structures, or, at all events, portions of them. He calls the upper node or pacemaker the right-sided, the lower node or commencement of the conduction system the left-sided remnant of a ring of special tissue which, in the lower vertebrate heart, surrounds the sino-auricular orifice. Our observations lend support to this view, for we find, that in the dog the right and left vagus nerves have in certain degree a selective action, each acting most potently upon that remnant which it is suggested is homolateral with it.

In conclusion, we would draw attention to the general bearing of these and similar observations upon the view that rhythmicity and conductivity are separate functions of the same heart muscle. The strict separation of these functions as a mental concept is essentially Engelmann's (5) and is dependent upon his experimental observation that vagal impulses, awakened reflexly, may show an effect upon one and not upon the other. It seems to us more probable, in the light of modern studies, that these selective effects are rather the result of changes in the direction taken by the efferent impulses, and that the efferent limb of the arc may be now the right, now the left inhibitory nerve. The action of the nerve impulse then is upon tissues happening to be specially endowed with certain attributes, a selective action of anatomical rather than of physiological significance.

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INDEX TO VOLUME XVIII.

ABSORPTION of adrenalin, relation of rate of, to its glycosuric and diuretic effects, 190
Acid, glycuronic, excretion of, in pneumococcus infections, with special reference to lobar pneumonia, 259
Acid media, growth of tissue in, 183
Acids, fatty, hemolytic properties of, and their relation to the causation of toxic hemolysis and pernicious anemia, 527
Adrenalin, relation of absorption of, to its glycosuric and diuretic effects, 190
Agglutinins for *Treponema pallidum*, 18
Albuminolysins (protein sensitizers) and precipitins, studies on the identity of, 219
Alcoholic extracts of tissues used for antigens in the Wassermann reaction, study of the addition of cholesterin to, 75
Amœbæ, cultivation of, in pure culture, upon autolyzed tissues, 252
Anaphylactic rabbit, an electrocardiographic study of, 450
Anaphylaxis and proteid cleavage products, relation between, 210
Anaphylaxis, serum, disturbances of the heart-beat in the dog caused by, 556
Anemia, blood flow in the hands in cases of, 113
Anemia, pernicious, and toxic hemolysis, hemolytic properties of fatty acids and their relation to the causation of, 527
Antigens in the Wassermann reaction, study of the addition of cholesterin to the alcoholic extracts of tissues used for, 75
AUER, JOHN, and ROBINSON, G. CANBY. An electrocardiographic study of the anaphylactic rabbit, 450
AUER, JOHN, and VAN SLYKE, DONALD D. A contribution to the relation between proteid cleavage products and anaphylaxis, 210
AUER, JOHN. See ROBINSON and AUER, 556
Auricle of the dog, relation of the auricular activity following faradization of, to abnormal auricular activity in man, 704
Auricles and ventricles in the dog, predominant influence of the left vagus nerve upon conduction between, 739
Auricular activity, abnormal, in man, and auricular activity following faradization of the dog's auricle, relation of, 704
Auricular activity following faradization of the dog's auricle, relation of, to abnormal auricular activity in man, 704
AUSTIN, J. HAROLD. See FARR and AUSTIN, 228
Autolyzed tissues, cultivation of amœbæ in pure culture upon, 252
Axis cylinders *in vitro*, regeneration of, 412

BACILLI, tubercle, intraperitoneal lysis of, 601
Bacillus megatherium, pneumonic lesions caused by, 543
Bactericidal action, selective, of methylene-blue, 187
Bladder, urinary, elimination through the mucosa of, 210
Blood destruction and regeneration and hemolytic jaundice, relation of the spleen to, 487, 494, 665
Blood flow in the feet, 354
Blood flow in the feet with special reference to fever, 372
Blood flow in the hands in cases of anemia, 113
Blood in rats recovered from implanted sarcoma, 390
Blood of splenectomized dogs, regeneration of, after the administration of hemolytic agents, 665
Blood, oxygen content of, in lobar pneumonia, 7
Blood, oxygen content of, in rabbits infected with pneumococcus, 1
Blood picture at various periods after splenectomy, 487

Blood picture of malaria, malarial pigment (hematin) as an active factor in the production of, 96
Blood platelets, histogenesis of, 278
Blood, total non-protein nitrogen of, in nephritis and allied conditions, 228
Bronchial and pulmonary circulation, relation of, 500
BRONFENBRENNER, JACOB. See MANWARING and BRONFENBRENNER, 601
BROWN, WADE H. Malarial pigment (hematin) as an active factor in the production of the blood picture of malaria, 96
——. The histogenesis of blood platelets, 278
BROWN, WADE H., and LOEVENHART, A. S. The effect of hematin on the circulation and respiration, 107
BUNTING, C. H., and JONES, A. P. Intestinal obstruction in the rabbit. II, 25

CANCER, experimental, mechanisms of metastasis formation in, 397
CARREL, ALEXIS. Concerning visceral organisms, 155
——. Contributions to the study of the mechanism of the growth of connective tissue, 287
Cells, implanted tumor, resistance to, as distinct from resistance to the tumor-producing agent, 416
Cerebrospinal fluid in acute poliomyelitis, 242
Cerebrospinal fluid in syphilis, 162
Cholesterin, study of the addition of, to the alcoholic extracts of tissues used for antigens in the Wassermann reaction, 75
CHURCHMAN, JOHN W. The selective bactericidal action of methylene-blue, 187
CHURCHMAN, JOHN W., and HERZ, LUCIUS FELIX. The toxicity of gentian violet and its fate in the animal body, 579
Circulation and respiration, effect of hematin on, 107
Circulation, bronchial and pulmonary, relation of, 500
Circulation in experimental pulmonary embolism, 507
Circulation in man, studies on, 113, 354, 372
Circulation of the kidney, mechanical obstruction to, produced by experimental acute toxic nephropathy, 29
Cleavage products, proteid, and anaphylaxis, relation between, 210
COHEN, MARTIN. See NOGUCHI and COHEN, 572
COHN, ALFRED E. The effect of morphin on the mechanism of the dog's heart after removal of one vagus nerve, 715
COHN, ALFRED E., and LEWIS, THOMAS. The predominant influence of the left vagus nerve upon conduction between the auricles and ventricles in the dog, 739
Conduction between auricles and ventricles in the dog, predominant influence of the left vagus nerve upon, 739
Connective tissue, mechanism of the growth of, 287
COURET, MAURICE, and WALKER, JAMES. The cultivation of amœbæ in pure culture upon autolyzed tissues, 252
Crotalus venom, experimental study of the late glomerular lesions caused by, 149
Cultivation of amœbæ in pure culture upon autolyzed tissues, 252
Cultivation of so called trachoma bodies, experiments on, 572
Cultivation of the microörganism causing epidemic poliomyelitis, experiments on, 461
Cultivation of the parasite of rabies, 314
Culture media, fluid and solid, comparative viability of pneumococci on, 584
Cylinders, axis, *in vitro*, regeneration of, 412

DIURETIC and glycosuric effects of adrenalin, relation of its rate of absorption to, 190

ELECTROCARDIOGRAPHIC study of the anaphylactic rabbit, 450
Elimination through the mucosa of the urinary bladder, 310
ELLIS, ARTHUR W. M., and SWIFT, HOMER F. The cerebrospinal fluid in syphilis, 162
——. The effect of intraspinous injections of salvarsan and neosalvarsan in monkeys, 428
ELLIS, ARTHUR W. M. See SWIFT and ELLIS, 435
Embolism, experimental pulmonary, circulation in, 507
Embryonic tissues *in vitro*, influence of temperature and fluid medium on the survival of, 406

Epidemic poliomyelitis, microörganism causing, experiments on the cultivation of, 461
Excretion of glycuronic acid in pneumococcus infections, with special reference to lobar pneumonia, 259
Experimental acute toxic nephropathy, mechanical obstruction to the circulation of the kidney produced by, 29
Experimental cancer, mechanisms of metastasis formation in, 397
Experimental nephritis, production of, by repeated proteid intoxication, 678
Experimental pneumonia in rabbits, 50, 61
Experimental pneumonia, rôle of the leucocyte in, and relation of the number of organisms injected to the mortality, 50
Experimental pulmonary embolism, circulation in, 507
Experimental studies in tetany, 618
Experimental study of the late glomerular lesions caused by crotalus venom, 149

FARADIZATION of the dog's auricle, relation of the auricular activity following, to abnormal auricular activity in man, 704
FARR, CLIFFORD B., and AUSTIN, J. HAROLD. The total non-protein nitrogen of the blood in nephritis and allied conditions, 228
Fatty acids, hemolytic properties of, and their relation to the causation of toxic hemolysis and pernicious anemia, 527
Ferment action, studies on, 591, 597
Fever, blood flow in the feet with special reference to, 372
Filterable cause, characters of a third transplantable chicken tumor due to, 651
FLEXNER, SIMON, and NOGUCHI, HIDEYO. Experiments on the cultivation of the microörganism causing epidemic poliomyelitis, 461
Fluid, cerebrospinal, in acute poliomyelitis, 242
Fluid, cerebrospinal, in syphilis, 162
Fluid medium and temperature, influence of, on the survival of embryonic tissues *in vitro*, 406
FRASER FRANCIS R. A study of the cerebrospinal fluid in acute poliomyelitis, 242

GENTIAN violet, toxicity of, and its fate in the animal body, 579
GHOREYEB, ALBERT A. A study of the mechanical obstruction to the circulation of the kidney produced by experimental acute toxic nephropathy, 29
GHOREYEB, ALBERT A., and KARSNER, HOWARD T. A study of the relation of pulmonary and bronchial circulation, 500
GHOREYEB, ALBERT A. See KARSNER and GHOREYEB, 507
GILLESPIE, L. J. The comparative viability of pneumococci on solid and on fluid culture media, 584
GITHENS, THOMAS STOTESBURY. The influence of temperature on the action of strychnin in frogs, 300
Glomerular lesions, late, caused by crotalus venom, experimental study of, 149
Glycosuric and diuretic effects of adrenalin, relation of its rate of absorption to, 190
Glycuronic acid, excretion of, in pneumococcus infections, with special reference to lobar pneumonia, 259
GOLDSCHMIDT, SAMUEL. See UNDERHILL, WELLS, and GOLDSCHMIDT, 317, 322, 347

HARTWELL, JOHN A. Intestinal obstruction, 139
Heart of the dog, effect of morphin on the mechanism of, after removal of one vagus nerve, 715
Heart-beat in the dog, disturbances of, caused by serum anaphylaxis, 556
Hematin, effect of, on the circulation and respiration, 107
Hematin (malarial pigment), as an active factor in the production of the blood picture of malaria, 96
Hemolysis, toxic, and pernicious anemia, hemolytic properties of fatty acids and their relation to the causation of, 527
Hemolytic agents, regeneration of the blood of splenectomized dogs after the administration of, 665
Hemolytic jaundice and blood destruction and regeneration, relation of the spleen to, 487, 494, 665
Hemolytic properties of fatty acids and their relation to the causation of toxic hemolysis and pernicious anemia, 527
Hemolytic serum, effect of, in splenectomized dogs, 494
HERZ, LUCIUS FELIX. See CHURCHMAN and HERZ, 579
Histogenesis of blood platelets, 278

IMMUNITY to experimental pneumonia in rabbits, 61
Implanted sarcoma, blood in rats recovered from, 390
Implanted tumor cells, resistance to, as distinct from resistance to the tumor-producing agent, 416
In vitro embryonic tissues, influence of temperature and fluid medium on the survival of, 406
In vitro regeneration of axis cylinders, 412
Infarction, studies in. III. The circulation in experimental pulmonary embolism, 507
Infections, pneumococcus, especially lobar pneumonia, excretion of glycuronic acid in, 259
INGEBRIGTSEN, RAGNVALD. Regeneration of axis cylinders *in vitro*, 412
Injections, intraspinous, of salvarsan and neosalvarsan in monkeys, 428
Insufflation, intrabronchial, of virulent streptococci, pneumonic lesions produced by, 548
Intestinal obstruction, 139
Intestinal obstruction in the rabbit. II, 25
Intoxication, proteid, repeated, production of experimental nephritis by, 678
Intrabronchial insufflation of virulent streptococci, pneumonic lesions produced by, 548
Intracanalicular pattern, sarcoma of, 651
Intraperitoneal lysis of tubercle bacilli, 601
Intraspinous injections of salvarsan and neosalvarsan in monkeys, 428

JAUNDICE, hemolytic, and blood destruction and regeneration, relation of the spleen to, 487, 494, 665
JOBLING, JAMES W., and STROUSE, SOLOMON. Studies on ferment action. VIII. The toxicity of some proteoses, 591
——. Studies on ferment action. IX. A note on the relation between lysis and proteolysis of pneumococci, 597
JONES, A. P. See BUNTING and JONES, 25

KARSNER, HOWARD T., and GHOREYEB, ALBERT A. Studies in infarction. III. The circulation in experimental pulmonary embolism, 507
KARSNER, HOWARD T. See GHOREYEB and KARSNER, 500
Kidney, mechanical obstruction to the circulation of, produced by experimental acute toxic nephropathy, 29
KLEINER, ISRAEL S. On elimination through the mucosa of the urinary bladder, 310
KLEINER, ISRAEL S., and MELTZER, S. J. The relation of the rate of absorption of adrenalin to its glycosuric and diuretic effects, 190
KLINE, B. S., and WINTERNITZ, M. C. Studies upon experimental pneumonia in rabbits. V. The rôle of the leucocyte in experimental pneumonia. The relation of the number of organisms injected to the mortality, 50
——. Studies upon experimental pneumonia in rabbits. VI. Studies in immunity, 61
KOLMER, JOHN A. Concerning agglutinins for *Treponema pallidum*, 18
KRUMBHAAR, EDWARD B., MUSSER, JOHN H., JR., and PEARCE, RICHARD M. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. VIII. Regeneration of the blood of splenectomized dogs after the administration of hemolytic agents, 665
KRUMBHAAR, EDWARD B. See MUSSER and KRUMBHAAR, 487

LAMBERT, ROBERT A. The influence of temperature and fluid medium on the survival of embryonic tissues *in vitro*, 406
LANGE, LINDA B. See ROUS and LANGE, 651
Lesions, late glomerular, caused by crotalus venom, experimental study of, 149
Lesions, pneumonic, caused by *Bacillus megatherium*, 543
Lesions, pneumonic, produced by intrabronchial insufflation of virulent streptococci, 548
Leucocyte, rôle of, in experimental pneumonia, and relation of the number of organisms injected to the mortality, 50
LEVIN, ISAAC. The mechanisms of metastasis formation in experimental cancer, 397
LEWIS, THOMAS. See COHN and LEWIS, 739
Lobar pneumonia and pneumococcus infections, excretion of glycuronic acid in, 259
Lobar pneumonia, oxygen content of the blood in, 7

LOEVENHART, A. S. See BROWN and LOEVENHART, 107
LONG, ESMOND R. The purines and purine metabolism of some tumors in domestic animals, 512
LONGCOPE, WARFIELD T. The production of experimental nephritis by repeated proteid intoxication, 678
Lysis and proteolysis of pneumococci, relation between, 597
Lysis, intraperitoneal, of tubercle bacilli, 601

MACCALLUM, W. G., and VOGEL, KARL M. Further experimental studies in tetany, 618
Malaria, blood picture of, malarial pigment (hematin) as an active factor in the production of, 96
Malarial pigment (hematin) as an active factor in the production of the blood picture of malaria, 96
MANWARING, WILFRED H., and BRONFENBRENNER, JACOB. On intraperitoneal lysis of tubercle bacilli, 601
MCPHEDRAN, WILLIAM FLETCHER. On the hemolytic properties of fatty acids and their relation to the causation of toxic hemolysis and pernicious anemia, 527
Media, acid, growth of tissue in, 183
Media, culture, fluid and solid, comparative viability of pneumococci on, 584
MEDIGRECEANU, FLORENTIN. On the excretion of glycuronic acid in pneumococcus infections, with special reference to lobar pneumonia, 259
Medium, fluid, and temperature, influence of, on the survival of embryonic tissue *in vitro*, 406
Megatherium, Bacillus, pneumonic lesions caused by, 543
MELTZER, S. J. See KLEINER and MELTZER, 190
——. See WOLLSTEIN and MELTZER, 543, 548
Metabolism, purine, and purines of some tumors in domestic animals, 512
Metastasis formation in experimental cancer, mechanisms of, 397
Methylene-blue, selective bactericidal action of, 187
Microörganism causing epidemic poliomyelitis, experiments on the cultivation of, 461
Morphin, effect of, on the mechanism of the dog's heart after removal of one vagus nerve, 715
Mucosa of the urinary bladder, elimination through, 310
MUSSER, JOHN H., JR., and KRUMBHAAR, EDWARD B. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. VI. The blood picture at various periods after splenectomy, 487
MUSSER, JOHN H., JR. See KRUMBHAAR, MUSSER, and PEARCE, 665

NEOSALVARSAN and salvarsan, intraspinous injections of, in monkeys, 428
Nephritis and allied conditions, total non-protein nitrogen of the blood in, 228
Nephritis, experimental, production of, by repeated proteid intoxication, 678
Nephritis, tartrate, renal secretion during, 347
Nephritis, tartrate, with especial reference to some of the conditions under which it may be produced, 322
Nephropathy, experimental acute toxic, mechanical obstruction to the circulation of the kidney produced by, 29
Nerve, vagus, effect of morphin on the mechanism of the dog's heart after removal of one, 715
Nitrogen, total non-protein, of the blood in nephritis and allied conditions, 228
NOGUCHI, HIDEYO. Contribution to the cultivation of the parasite of rabies, 314
NOGUCHI, HIDEYO, and COHEN, MARTIN. Experiments on the cultivation of the so called trachoma bodies, 572
NOGUCHI, HIDEYO. See FLEXNER and NOGUCHI, 461
Non-protein nitrogen, total, of the blood in nephritis and allied conditions, 228

OBSTRUCTION, intestinal, 139
Obstruction, intestinal, in the rabbit. II, 25
Obstruction, mechanical, to the circulation of the kidney produced by experimental acute toxic nephropathy, 29
Oxygen content of the blood in lobar pneumonia, 7
Oxygen content of the blood in rabbits infected with pneumococcus, 1

PALLIDUM, *Treponema*, agglutinins for, 18
Parasite of rabies, cultivation of, 314
PEABODY, FRANCIS W. The oxygen content of the blood in rabbits infected with pneumococcus, 1
——. The oxygen content of the blood in lobar pneumonia, 7
PEARCE, RICHARD M. An experimental study of the late glomerular lesions caused by crotalus venom, 149
PEARCE, RICHARD M., and PEET, MAX MINOR. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. VII. The effect of hemolytic serum in splenectomized dogs, 494
PEARCE, RICHARD M. See KRUMBHAAR, MUSSER, and PEARCE, 665
PEET, MAX MINOR. See PEARCE and PEET, 494
Pigment, malarial, (hematin), as an active factor in the production of the blood picture of malaria, 96
Platelets, blood, histogenesis of, 278
Pneumococci, comparative viability of, on solid and on fluid culture media, 584
Pneumococci, relation between lysis and proteolysis of, 597
Pneumococcus infections, especially lobar pneumonia, excretion of glycuronic acid in, 259
Pneumococcus, oxygen content of the blood in rabbits infected with, 1
Pneumonia, experimental, in rabbits, 50, 61
Pneumonia, experimental, rôle of the leucocyte in, and relation of the number of organisms injected to the mortality, 50
Pneumonia, lobar, and pneumococcus infections, excretion of glycuronic acid in, 259
Pneumonia, lobar, oxygen content of the blood in, 7
Pneumonic lesions caused by *Bacillus megatherium*, 543
Pneumonic lesions produced by intrabronchial insufflation of virulent streptococci, 548
Poliomyelitis, acute, cerebrospinal fluid in, 242
Poliomyelitis, epidemic, microörganism causing, experiments on the cultivation of, 461
Precipitins and protein sensitizers (albuminolysins), studies on the identity of, 219
Proteid cleavage products and anaphylaxis, relation between, 210
Proteid intoxication, repeated, production of experimental nephritis by, 678
Protein sensitizers (albuminolysins) and precipitins, studies on the identity of, 219
Proteolysis and lysis of pneumococci, relation between, 597
Proteoses, toxicity of some, 591
Pulmonary and bronchial circulation, relation of, 500
Pulmonary embolism, experimental, circulation in, 507
Purine metabolism and purines of some tumors in domestic animals, 512
Purines and purine metabolism of some tumors in domestic animals, 512

RABIES, cultivation of the parasite of, 314
Reaction, Wassermann, study of the addition of cholesterin to the alcoholic extracts of tissues used for antigens in, 75
Regeneration of axis cylinders *in vitro*, 412
Renal secretions during tartrate nephritis, 347
Respiration and circulation, effect of hematin on, 107
ROBINSON, G. CANBY. The relation of the auricular activity following faradization of the dog's auricle to abnormal auricular activity in man, 704
ROBINSON, G. CANBY, and AUER, JOHN. Disturbances of the heart-beat in the dog caused by serum anaphylaxis, 556
ROBINSON, G. CANBY. See AUER and ROBINSON, 450
ROUS, PEYTON. The growth of tissue in acid media, 183
——. Resistance to a tumor-producing agent as distinct from resistance to the implanted tumor cells, 416
ROUS, PEYTON, and LANGE, LINDA B. The characters of a third transplantable chicken tumor due to a filterable cause. A sarcoma of intracanalicular pattern, 651

SALVARSAN and neosalvarsan, intraspinous injections of, in monkeys, 428
Salvarsan, spirochæticidal action of the serum of patients treated with, 435
Sarcoma, implanted, blood in rats recovered from, 300

Sarcoma of intracanalicular pattern, 651
Secretions, renal, during tartrate nephritis, 347
Sensitizers, protein (albuminolysins), and precipitins, studies on the identity of, 219
Serum anaphylaxis, disturbances of the heart-beat in the dog caused by, 556
Serum, hemolytic, effect of, in splenectomized dogs, 494
Serum of patients treated with salvarsan, spirochæticidal action of, 435
Spirochæticidal action of the serum of patients treated with salvarsan, study of, 435
Spleen, relation of, to blood destruction and regeneration and to hemolytic jaundice, 487, 494, 665
Splenectomized dogs, effect of hemolytic serum in, 494
Splenectomized dogs, regeneration of blood of, after the administration of hemolytic agents, 665
Splenectomy, blood picture at various periods after, 487
STEWART, G. N. Studies on the circulation in man. VI. Observations on the blood flow in the hands (mainly) in cases of anemia, 113
——. Studies on the circulation in man. VII. The blood flow in the feet, 354
——. Studies on the circulation in man. VIII. The blood flow in the feet with special reference to fever, 372
Streptococci, virulent, pneumonic lesions produced by intrabronchial insufflation of, 548
STROUSE, SOLOMON. See JOBLING and STROUSE, 591, 597
Strychnin in frogs, influence of temperature on the action of, 300
SWIFT, HOMER F., and ELLIS, ARTHUR W. M. A study of the spirochæticidal action of the serum of patients treated with salvarsan, 435
SWIFT, HOMER F. See ELLIS and SWIFT, 162, 428
——. See WALKER and SWIFT, 75
Syphilis, cerebrospinal fluid in, 162

TARTRATE nephritis, renal secretions during, 347
Tartrate nephritis, with especial reference to some of the conditions under which it may be produced, 322
Tartrates, fate of, in the body, 317
Temperature and fluid medium, influence of, on the survival of embryonic tissues *in vitro*, 406
Temperature, influence of, on the action of strychnin in frogs, 300
Tetany, further experimental studies in, 618
Tissue, connective, mechanism of the growth of, 287
Tissue, growth of, in acid media, 183
Tissues, alcoholic extracts of, used for antigens in the Wassermann reaction, study of the addition of cholesterin to, 75
Tissues, autolyzed, cultivation of amœbæ in pure culture upon, 252
Tissues, embryonic, *in vitro*, influence of temperature and fluid medium on the survival of, 406
Toxicity of some proteoses, 591
Trachoma bodies, so called, experiments on the cultivation of, 572
Transplantable chicken tumor, third, due to a filterable cause, characters of, 651
Treponema pallidum, agglutinins for, 18
Tubercle bacilli, intraperitoneal lysis of, 601
Tumor cells, implanted, resistance to, as distinct from resistance to the tumor-producing agent, 416
Tumor of chicken, third transplantable, due to a filterable cause, characters of, 651
Tumor-producing agent, resistance to, as distinct from resistance to the implanted tumor cells, 416
Tumors in domestic animals, the purines and purine metabolism of, 512

UNDERHILL, FRANK P., WELLS, H. GIDEON, and GOLDSCHMIDT, SAMUEL. A note on the fate of tartrates in the body, 317
——. Tartrate nephritis, with especial reference to some of the conditions under which it may be produced, 322
——. A study of renal secretions during tartrate nephritis, 347
Urinary bladder, elimination through the mucosa of, 310

VAGUS nerve, effect of morphin on the mechanism of the dog's heart after removal of one, 715
Vagus nerve, left, predominant influence of, upon conduction between

auricles and ventricles in the dog, 739
VAN SLYKE, DONALD D. See AUER and VAN SLYKE, 210
Venom, crotalus, experimental study of the late glomerular lesions caused by, 149
Ventricles and auricles in the dog, predominant influence of the left vagus nerve upon conduction between, 739
Viability of pneumococci, comparative, on solid and on fluid culture media, 584
Violet, gentian, toxicity of, and its fate in the animal body, 579
Visceral organisms, 155
VOGEL, KARL M. See MACCALLUM and VOGEL, 618

WALKER, I. C., and SWIFT, HOMER F. A study of the addition of cholesterin to the alcoholic extracts of tissues used for antigens in the Wassermann reaction, 75
WALKER, JAMES. See COURET and WALKER, 252
Wassermann reaction, study of the addition of cholesterin to the alcoholic extracts of tissues used for antigens in, 75
WEIL, RICHARD. A study of the blood in rats recovered from implanted sarcoma, 390
WELLS, H. GIDEON. See UNDERHILL, WELLS, and GOLDSCHMIDT, 317, 322, 347
WINTERNITZ, M. C. See KLINE and WINTERNITZ, 50, 61
WOLLSTEIN, MARTHA, and MELTZER, S. J. Pneumonic lesions caused by *Bacillus megatherium*, 543
——. The character of the pneumonic lesions produced by intrabronchial insufflation of virulent streptococci, 548

ZINSSER, HANS. Further studies on the identity of precipitins and protein sensitizers (albuminolysins), 219

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Monograph No. 2.

(Issued February 15, 1911)

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Monograph No. 3.

(Issued March 15, 1911)

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Monograph No. 4.

(Issued June 24, 1912)

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CONTENTS.

MANWARING, WILFRED H., and BRONFENBRENNER, J. On Intraperitoneal Lysis of Tubercle Bacilli 601

MACCALLUM, W. G., and VOGEL, KARL M. Further Experimental Studies in Tetany 618

ROUS, PEYTON, and LANGE, LINDA B. The Characters of a Third Transplantable Chicken Tumor Due to a Filterable Cause. A Sarcoma of Intracanalicular Pattern. Plates 49 to 55 . . 651

KRUMBHAAR, EDWARD B., MUSSER, JOHN H., Jr., and PEARCE, RICHARD M. The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. VIII. Regeneration of the Blood of Splenectomized Dogs after the Administration of Hemolytic Agents 665

LONGCOPE, WARFIELD T. The Production of Experimental Nephritis by Repeated Proteid Intoxication. Plates 56 to 65 678

ROBINSON, G. CANBY. The Relation of Auricular Activity Following Faradization of the Dog's Auricle to Abnormal Auricular Activity in Man. Plates 66 to 68 704

COHN, ALFRED E. The Effect of Morphin on the Mechanism of the Dog's Heart after Removal of One Vagus Nerve. Plates 69 to 73 715

COHN, ALFRED E., and LEWIS, THOMAS. The Predominant Influence of the Left Vagus Nerve upon Conduction between Auricles and Ventricles in the Dog. Plate 74 . . . 739

Index to Volume XVIII 748

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1 3 2 E
2 5 E 3
3 3 2 E
4 2 8 3
5 5 8 2
6 E 3 8